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European Patent Office

Office européen des brevets



Publication number: **0 550 296 A2**

(12)

## EUROPEAN PATENT APPLICATION

(21) Application number: **92403199.0**

(22) Date of filing: **27.11.92**

(51) Int. Cl.<sup>5</sup>: **C12N 15/18, C07K 13/00,  
C12P 21/02, C12N 5/10,  
A61K 37/36**

A request for correction of figure 12 and page 8 and a request for addition of a missing word on the fourth line from the bottom of page 33 has been filed pursuant to Rule 88 EPC. A decision on the request will be taken during the proceedings before the Examining Division (Guidelines for Examination in the EPO, A-V, 2.2).

The application is published incomplete as filed (Article 93 (2) EPC). The point in the description at which the omission obviously occurs has been left blank.

(30) Priority: **28.11.91 JP 337999/91**

(43) Date of publication of application:  
**07.07.93 Bulletin 93/27**

(84) Designated Contracting States:  
**BE DE FR GB IT NL SE**

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(54) **Vascular endothelial cells growth factor.**

(57) A novel protein of human origin produced by a human ovarian tumor established cell line HUOCA-II or HUOCA-III, which has a molecular weight, when determined by SDS-polyacrylamide gel electrophoresis, of from 72,000 to 80,000 daltons under a non-reducing condition or from 79,000 to 85,000 daltons under a reducing condition, which contains an amino acid sequence represented by the Sequence ID No. 4 deduced from the DNA sequence represented by the Sequence ID No. 5, and which enhances growth of vascular endothelial cells but does not activate growth of smooth muscle cells, fibroblasts and hepatocytes and also does not enhance or inhibit growth of HeLa cells. This invention also provides a process for the production of the protein.

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FIELD OF THE INVENTION

This invention relates to a novel protein of human origin and its production process. Particularly, it relates to a novel proteinous angiogenic factor of human origin, which enhances the growth of vascular endothelial cells but does not activate the growth of other cells such as smooth muscle cells, fibroblasts, hepatocytes and the like, and to a process for the production thereof.

BACKGROUND OF THE INVENTION

Principal cells which constitute a blood vessel are vascular endothelial cells of tunica intima, smooth muscle cells of tunica media and fibroblasts of tunica externa. In addition, peripherally existing capillary blood vessels are composed solely of vascular endothelial cells. Though the mechanism of new formation of blood vessels, or angiogenesis, has not yet been elucidated in full details, it is considered that the angiogenesis starts firstly with dissolution of the blood vessel wall matrix and subsequent growth and migration of vascular endothelial cells.

Angiogenesis can be found during the prenatal period when new tissues and blood vessels are formed and at the time of the occurrence of physiological phenomena in the adult body such as periodical development of uterine endometrium and lutenization in ovaries, as well as under pathologic conditions such as chronic inflammation, wound healing and the like. New formation of blood vessels can also be found at the time of the growth of tumor cells. Endothelial cells which cover the inner wall of blood vessels are possessed of many physiological functions such as maintenance of anti-thrombotic activity, regulation of matter permeation, regulation of blood pressure and the like. In a patient suffering from a blood vessel-related disease such as arteriosclerosis, myocardial infarction or the like, abnormality can be found in these blood vessel-constituting cells.

A number of angiogenic factors have been found in the *in vivo* experimental systems for the formation of new blood vessels, such as an experiment in which chick chorio-allantoic membrane is used. For example, generally known proteinous angiogenic factors include basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF) and the like.

Though these prior art angiogenic factors having the ability to enhance formation of new blood vessels are possessed of the activity to enhance growth of vascular endothelial cells, these factors also strongly activate growth of other cells. For example, bFGF activates growth of various cells such as fibroblasts, smooth muscle cells, epidermal cells and the like. In consequence, each of these prior art angiogenic factors having a broad range of growth enhancing effects on various types of cells enhances not only the formation of new blood vessels but also the growth of other cells at the same time. In other words, these prior art factors have a problem of causing secondary reactions when used because of their inability to selectively enhance formation of new blood vessels.

Accordingly, the present invention contemplates overcoming the aforementioned problems involved in the prior art and, as the results, providing a purified angiogenic factor which enhances growth of vascular endothelial cells but does not or hardly activate growth of other cells such as smooth muscle cells, fibroblasts, hepatocytes and the like. The present invention also contemplates developing side effect-free pharmaceutical preparations and medical devices based on such a purified angiogenesis factor.

With the aim of accomplishing these objects, the inventors of the present invention have conducted intensive studies and found that products of human ovarian tumor established cell lines, HUOCA-II and HUOCA-III, were able to enhance growth of vascular endothelial cells selectively. The results have been disclosed in Japanese Patent Application Kokai Nos. 2-261375, 2262523 and 3-84000.

Thereafter, the present inventors have carried out studies on the purification of the aforementioned products of HUOCA-II and HUOCA-III cell lines from their serum-free culture supernatants, making use of specific purification techniques, and have succeeded in obtaining a highly purified specific protein having the aforementioned desirable properties, that is, having a strong activity to enhance growth of vascular endothelial cells but with no activity to activate growth of other cells such as smooth muscle cells, fibroblasts, hepatocytes and the like.

By further continuing the studies, a total RNA was isolated from the HUOCA-II or HUOCA-III cells and its cDNA was cloned. Thereafter, the DNA sequence of the cDNA was determined and its corresponding amino acid sequence was deduced, thereby succeeding in obtaining the novel protein of the present invention.

SUMMARY OF THE INVENTION

According to a first aspect of the present invention, there is provided a single chain protein produced by

HUOCA-II or HUOCA-III, which has the following properties of:

- (1) having a molecular weight, when determined by SDS polyacrylamide gel electrophoresis, of from 72,000 to 80,000 daltons under a non-reducing condition or from 79,000 to 85,000 daltons under a reducing condition;
- 5 (2) containing three peptide chains, respectively represented by the Sequence ID Nos. 1, 2 and 3 as attached hereto (in the Sequence ID No. 3, "Xaa" means an unidentified amino acid residue), in one molecule;
- (3) having an activity to enhance the growth of vascular endothelial cells;
- (4) having no activity to enhance the growth of fibroblasts; vascular smooth muscle cells and hepatocytes;
- 10 (5) having no activity to enhance or inhibit the growth of HeLa cells; and
- (6) having an activity to enhance formation of new blood vessels.

According to a second aspect of the present invention, there is provided a protein of human origin which contains an amino acid sequence or a portion of the amino acid sequence represented by the Sequence ID No. 4 attached hereto that has been identified by isolating a corresponding total RNA molecule from HUOCA-II or HUOCA-III cells, cloning a cDNA corresponding to the proteins, determining the DNA sequence of the cDNA and deducing an amino acid sequence from the DNA sequence.

According to a third aspect of the present invention, there is provided a process for the production of a protein of human origin according to the first or second aspect of the present invention, which comprises purifying a serum-free culture supernatant of a human ovarian tumor cell or established cell line thereof, especially HUOCA-II or HUOCA-III, by an optional combination of purification techniques including (a) cation exchange chromatography, (b) heparin affinity chromatography, (c) heparin affinity high performance liquid chromatography and (d) reverse phase high performance liquid chromatography, or which comprises the steps of (i) preparing a DNA fragment containing a nucleotide sequence which encodes the protein or a portion of the protein shown in the Sequence ID No. 4 attached hereto, (ii) obtaining a transformant by transforming cells of a host with the DNA fragment prepared in the above step (i) or with a vector containing the DNA fragment and (iii) culturing the transformant obtained in the above step (ii) to allow the transformant to produce the protein of the Sequence ID No. 4, or a portion of the protein, subsequently recovering the protein from resulting culture mixture.

According to a fourth aspect of the present invention, there is provided a pharmaceutical preparation which contains the protein or a portion of the protein of the first and/or second aspect of the present invention as an active ingredient

According to a fifth aspect of the present invention, there is provided a DNA fragment or cDNA-fragment which contains a nucleotide sequence or a portion of the nucleotide sequence represented by the Sequence ID No. 5 attached hereto wherein at least one base may be substituted based on the degeneracy of genetic code.

According to a sixth aspect of the present invention, there is provided an expression vector containing the DNA fragment, as well as a transformant transformed with the DNA fragment or the expression vector.

Other objects and advantages of the present invention will be made apparent as the description progresses.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph showing the absorbance, measured at a wave length of 280 nm, of each eluate fraction resulting from the treatment of an HUOCA-III serum-free culture supernatant with cation exchange chromatography.

Fig. 2 is a graph showing the results of the measurement of activities in the eluate fractions obtained in Fig. 1 to enhance the growth of vascular endothelial cells.

Fig. 3 is a graph showing the absorbance, measured at a wave length of 280 nm, of each eluate fraction resulting from a heparin affinity chromatographic treatment of the active fractions of the cation exchange chromatography eluates having the vascular endothelial cell growth-enhancing activity.

Fig. 4 is a graph showing the results of the measurement of activities in the eluate fractions obtained in Fig. 3 to enhance the growth of vascular endothelial cells.

Fig. 5 is a graph showing the absorbance, measured at a wave length of 215 nm, of each eluate fraction resulting from a heparin affinity high performance liquid chromatographic treatment of the active fractions of the heparin affinity chromatography eluates having the vascular endothelial cell growth-enhancing activity.

Fig. 6 is a graph showing the results of the measurement of activities in the eluate fractions obtained in Fig. 5 to enhance growth of vascular endothelial cells.

Fig. 7 is a graph showing the absorbance, measured at a wave length of 215 nm, of each eluate fraction

resulting from a reverse phase high performance liquid chromatographic treatment of the active fractions of the heparin affinity high performance liquid chromatography eluates having the vascular endothelial cell growth-enhancing activity.

Fig. 8 is a graph showing the results of the measurement of activities in the eluate fractions obtained in Fig. 7 to enhance the growth of vascular endothelial cells.

Fig. 9 is a graph showing an SDS polyacrylamide gel electrophoresis pattern of a highly purified product (glycoprotein) obtained in Example 1 of the present invention.

Fig. 10 is a graph showing results of the measurement of the vascular endothelial cell growth-enhancing activity of the highly purified product eluted from each cut portion of the electrophoresis gel of Fig. 9.

Fig. 11 is a graph showing an SDS-polyacrylamide gel electrophoresis pattern of an N-glycanase-treated product of the highly purified product (glycoprotein) obtained in Example 1 of the present invention.

Fig. 12 represents the nucleotide sequence of the mRNA from which the cDNA obtained in Example 1 step (B) is translated and the corresponding amino acid sequence deduced from the nucleotide sequence.

## DETAILED DESCRIPTION OF THE INVENTION

Firstly, a first and a second aspects of the present invention are described in detail.

The gist of the first aspect of the present invention resides in a single chain protein produced by HUOCA-II or HUOCA-III, which has the following properties of:

- (1) having a molecular weight, when determined by SDS polyacrylamide gel electrophoresis, of from 72,000 to 80,000 daltons under a non-reducing condition or from 79,000 to 85,000 daltons under a reducing condition;
- (2) containing three peptide chains, respectively represented by the Sequence ID Nos. 1, 2 and 3 as attached hereto (in the Sequence ID No. 3, "Xaa" means an unidentified amino acid residue), in one molecule;
- (3) having an activity to enhance the growth of vascular endothelial cells;
- (4) having no activity to enhance the growth of fibroblasts, vascular smooth muscle cells and hepatocytes;
- (5) having no activity to enhance or inhibit the growth of HeLa cells; and
- (6) having an activity to enhance the formation of new blood vessels.

The gist of the second aspect of the present invention resides in a protein of human origin which contains an amino acid sequence or a portion of the sequence represented by the Sequence ID No. 4 attached hereto that has been identified by isolating a corresponding mRNA molecule from HUOCA-II or HUOCA-III cells, cloning a gene corresponding to the mRNA, determining the DNA sequence of the gene and deducing an amino acid sequence from the DNA sequence.

The human ovarian tumor established cell lines HUOCA-II and HUOCA-III have been deposited by the present inventors on March 1, 1989, in Fermentation Research Institute, Agency of Industrial Science and Technology, and have been assigned the designations as FERM BP-2310 and FERM BP-2311. Though culturing of the HUOCA-II and HUOCA-III and preparation of their serum-free culture supernatants may be carried out in the usual way, these techniques are disclosed in detail by the present inventors in Japanese Patent Application Kokai Nos. 2-261375, 2-262523 and 3-84000.

The protein of the present invention comprises a single chain protein molecule, and the single chain protein contains three peptide chains respectively represented by the Sequence ID Nos. 1, 2 and 3 as attached hereto.

The protein of the present invention may be prepared from a serum-free culture supernatant of the human ovarian tumor established cell line, HUOCA-II or HUOCA-III, by subjecting the supernatant to a series of purification steps including (a) cation exchange chromatography, (b) heparin affinity chromatography, (c) heparin affinity high-performance liquid chromatography and (d) reverse-phase high-performance liquid chromatography. Preferably, it may be prepared in accordance with the following illustrative steps (i) to (iv).

### Preparation of protein

(i) A serum-free culture supernatant of HUOCA-II or HUOCA-III is adsorbed on to a cation exchange resin packed in a column. In this instance, the cation exchange resin may be either strongly ionic or weakly ionic, but the use of S-Sepharose® (trademark of Pharmacia) is particularly preferred. The thus adsorbed portion onto a cation exchange resin in the column is washed with an appropriate buffer solution and then subjected to a linear gradient elution using two buffer solutions respectively containing 150 mM NaCl and 2 M NaCl to collect active fractions showing the activity to enhance the growth of vascular endothelial cells [step (a)].

(ii) The active fractions obtained in the above step (i) are pooled and diluted by a factor of 2 to 3 with the

same buffer solution containing 150 mM of NaCl. The thus diluted sample is applied to a heparin-Sepharose column, washed with the same buffer solution containing 0.5 M NaCl and then subjected to a linear gradient elution using two buffer solutions respectively containing 0.5 M NaCl and 2 M NaCl to collect active fractions showing the activity to enhance the growth of vascular endothelial cells [step (b)].

(iii) The active fractions obtained in the above step (ii) are diluted in the same manner, applied to a heparin column for high performance liquid chromatography use and then subjected to elution in the same manner to collect active fractions showing the activity to enhance the growth of vascular endothelial cells [step (c)].

(iv) The active fractions obtained in the above step (iii) are applied to a column for reverse-phase high-performance liquid chromatography use to obtain a purified product (protein) having the activity to enhance the growth of vascular endothelial cells [step (d)].

Any usually used buffer solution such as a phosphate buffer or the like may be used in the above glycoprotein preparation steps, and Sepharose or any other general purpose carrier may be used as a carrier of heparin.

The thus purified product has been identified as a glycoprotein, namely a sugar chain-attached protein molecule, on the basis of the facts that (1), when the purified product was allowed to react with a sugar chain-hydrolyzing enzyme *N*-glycanase and the resulting product was analyzed by 0.1% SDS-containing 10% polyacrylamide gel electrophoresis, the electrophoresis pattern of the thus treated product showed a decreased molecular weight level due to the digestion of sugar chains and (2) the purified product showed an affinity for concanavalin A.

In addition, the protein portion of the glycoprotein of the present invention was identified as a single chain protein molecule, because the purified product showed a single band when analyzed by 0.1% SDS-containing 10% polyacrylamide gel electrophoresis under reducing conditions.

Though the amino acid sequence of the protein portion of the thus obtained glycoprotein could be determined by any usually used means, the following illustrative steps (1) to (3) were employed herein in that order.

#### Determination of amino acid sequence

##### (1) Reductive carboxymethylation

The sample purified and isolated in the aforementioned step (iv) by reverse-phase high-performance liquid chromatography was concentrated using a concentrator and eluted with an eluting solution consisting of 8 M urea, 0.5 M Tris-HCl pH 8.0 and 1 mM EDTA. To this was added dithiothreitol to a final concentration of 20 mM. After nitrogen gas flush, the reduction reaction was carried out in the dark for 2 hours at room temperature. Thereafter, moniodoacetic acid was added to the resulting reaction mixture to a final concentration of 20 mM, and the alkylation reaction was carried out in the dark for 30 minutes at room temperature.

##### (2) Digestion with lysyl endopeptidase

The reductive alkylation product obtained in the above step (1) was mixed with 2-mercaptoethanol, followed by the addition of 0.1 N NaOH to adjust the mixture to pH 8.5. Lysyl endopeptidase (Wako Pure Chemical Industries, Ltd.) was added in a 1:10 (w/w) ratio to the thus prepared substrate to carry out the enzymatic hydrolysis reaction at 37°C for 4 hours.

##### (3) Fractionation of peptide fragments and determination of the amino acid sequence

The peptide fragments mixture obtained in the above step (2) were separated by reverse-phase high-performance chromatography using an RP300 column (Applied Biosystems, Inc.). The elution was carried out by linear concentration gradient of acetonitrile from 0% to 60% in the presence of 0.1% TFA. The thus obtained peptide fragments by the elution treatment were subjected to Edman degradation using a gas phase sequencer (Model 477A; Applied Biosystems, Inc.), and the resulting PTH-amino acids were identified using a high-performance liquid chromatography for PTH-amino acid identification use (Model 120A; Applied Biosystems, Inc.). As the results, it was found that the protein portion of the glycoprotein of the present invention contained three peptide chains respectively represented by the Sequence ID Nos. 1, 2 and 3.

#### Determination of the complete DNA sequence by PCR

The amino acid sequence determined in the above step (3) coincided well with that of human hepatocyte

growth factor (hHGF). With regard to hHGF, its cDNA sequence has been reported by Nakamura (*Nature*, vol.342, pp.440 - 443, 1989) and Miyazawa (*Biochemical and Biophysical Research Communication*, vol.163, pp.967 - 973, 1989).

Since several cDNA nucleotide sequences have been reported on the hHGF family, primers for PCR use were prepared using a DNA synthesizer based on the common sequences in the 5' and 3' non-translation regions of these known nucleotide sequences. That is, primers were synthesized based on a region including 47 to 82 position bases (5' primer) counting in upstream direction from the 5' end of the translation region (translation initiation point) and another region including 1 to 37 position bases (3' primer) counting in downstream direction from the 3' end.

The total RNA sample was prepared from the human ovarian tumor cell line HUOCA-III by means of an SDS-phenol method. Using the thus prepared total RNA as a template, cDNA synthesis was carried out making use of M-MLV reverse transcriptase. The thus synthesized cDNA was subjected to PCR and the resulting PCR product was applied to agarose gel electrophoresis to find a DNA fragment having a size of about 2.3 kb. Since the open reading frame of the HGF family so far reported has a size of about 2.3 kb, this DNA fragment was considered to be a cDNA molecule coding for the HUOCA-III-originated novel protein of the present invention. In consequence, this DNA fragment was purified from the agarose gel, inserted into the pUC18 plasmid vector and then transformed into *Escherichia coli* JM109. Some of the thus obtained clones were examined making use of the dideoxy method to determine their nucleotide sequences. By correcting reading errors at the time of the PCR study, a nucleotide sequence corresponding to the novel protein of HUOCA-III origin was determined. The thus determined nucleotide sequence is shown in the Sequence ID No. 5 attached hereto, and an amino acid sequence deduced from the nucleotide sequence in the Sequence ID No. 4

#### Measurement of molecular weight by SDS-polyacrylamide gel electrophoresis

Electrophoresis was carried out using a 10% polyacrylamide gel in accordance with the procedure of Lam-meli *et al.* (*Nature*, vol.277, pp.680 - 685, 1970). The resulting gel was fixed by treating it with 50% ethanol and 40% acetic acid for 30 minutes, washed with 10% ethanol and 5% acetic acid and then subjected to silver staining. The protein of the present invention was stained as a single band, and its molecular weight was estimated to be about 72,000 to 80,000 daltons based on its relative mobility. In addition, another electrophoresis was carried out under a reducing condition by adding 2-mercaptoethanol to the sample to a concentration of 5% and treating the mixture at 95°C for 10 minutes, followed by the same procedure as the case of the above non-reducing condition. Under the reducing condition, the molecular weight of the protein of the present invention was estimated to be about 79,000 to 85,000 daltons.

Next, a third aspect of the present invention is described in the following.

The gist of the third aspect of the present invention resides in a process for the production of the protein of the first or second aspect of the present invention.

Firstly, a culture mixture containing the protein of the first or second aspect of the present invention is obtained.

The single chain protein of the first aspect of the present invention is obtained by recovering it from a serum-free culture supernatant of the human ovarian tumor cell line, HUOCA-II or HUOCA-III

The novel protein of the second aspect of the present invention is obtained by preparing a DNA fragment containing a nucleotide sequence which encodes the novel protein represented by the amino acid sequence or a portion of the sequence shown in the Sequence ID No. 4, preferably the DNA fragment or a portion of the DNA fragment represented by the Sequence ID No. 5, transforming appropriate host cells with the thus ligated fragment directly or indirectly using a proper expression vector, culturing the thus obtained transformant and then recovering the novel protein of the Sequence ID No. 4 from the resulting culture mixture.

The recovering step may be effected, though not particularly limited, by purifying the novel protein by means of (a) cation exchange chromatography, (b) heparin affinity chromatography, (c) heparin affinity high-performance liquid chromatography and (d) reverse-phase high-performance liquid chromatography, in any optional combination or order.

According to a fourth aspect of the present invention, there is provided a pharmaceutical preparation which contains the protein of the first and/or second aspect of the present invention as an active ingredient.

The pharmaceutical preparation may be applied to various dosage forms such as tablets, sugar coated tablets, powders, capsules, granules, suspensions, emulsions, parenteral solutions, external preparations, ointments and the like, using the preparation alone or together with other necessary ingredients in combination with appropriate carriers, fillers and the like.

The protein of the present invention is possessed of a function to enhance vascular endothelial cell growth in human and various animals, but does not enhance the growth of fibroblasts, vascular smooth muscle cells

or hepatocytes in human and animals and does not enhance or inhibit the growth of HeLa cells. Because of such nature, the growth of vascular endothelial cells can be enhanced selectively and, as the results, new formation of blood vessels can be effected smoothly without causing secondary reactions.

The term "it does not enhance the growth of fibroblasts; vascular smooth muscle cells or hepatocytes and does not enhance or inhibit the growth of HeLa cells" as used herein includes two cases; one case meaning that it does not enhance the growth of fibroblasts, vascular smooth muscle cells or hepatocytes and does not enhance or inhibit the growth of HeLa cells at all, and the other case meaning that it shows these activities to some extent but to an extremely small degree in comparison with its activity to enhance the growth of vascular endothelial cells.

Illustrative procedures for the measurement of activities of the protein of the present invention to enhance the growth of vascular endothelial cells, fibroblasts, vascular smooth muscle cells, hepatocytes and HeLa cells and to inhibit the growth of HeLa cells will be described later in detail in Examples.

In addition to the above properties, the protein of the present invention shows an affinity for concanavalin A. In the present invention, the affinity for concanavalin A was examined in the following manner.

#### Measurement of affinity for concanavalin A

Using a dot blot apparatus (BioDot; Bio-Rad Laboratories, Inc.), a 500 ng portion of the purified product described in the foregoing was adsorbed to a nitrocellulose membrane (Bio-Rad Laboratories, Inc.) which has in advance been soaked in 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl. After air-drying, the resulting membrane was washed by soaking it for 10 minutes in 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl and 0.05% Tween and then replacing the washing buffer by a fresh one. After repeating the washing step 4 times, the membrane was soaked for 1 hour at 4°C in the same buffer which has been further supplemented with 1% BSA (bovine serum albumin), and washed again.

The thus treated membrane was soaked in a solution containing 10 µg/ml of labelled horseradish peroxidase (HRP) - concanavalin A at 4°C for 1 hour and washed again. Thereafter, the HRP remaining after the washing was allowed to perform a coloring reaction in the presence of H<sub>2</sub>O<sub>2</sub> using 3,3'-diaminobenzidine as a substrate, in order to judge the affinity of the inventive protein for concanavalin A. As the results, the purified product blotted on the membrane showed development of a brown color, while a control test resulted in no coloration, thus confirming the affinity of the purified product for concanavalin A.

As described in the foregoing, the protein of the present invention is possessed of excellent ability to enhance vascular endothelial cells growth as well as its function to enhance new formation of blood vessels. Because of such nature, a physiologically active pharmaceutical preparation containing the inventive protein can be used as a healing enhancer of wound, burn injury, decubitus, postoperative tissue damage or the like or as a drug for the treatment of cardiac angiopathy, as well as its application to artificial organs such as artificial blood vessel, artificial skin and the like. In addition, antibodies specific for the protein of the present invention and inhibitors of the inventive protein can be used effectively as diagnostic and therapeutic drugs of malignant tumor, retinopathy, chronic rheumatoid arthritis and the like.

#### EXAMPLES

The following examples are provided to further illustrate the preparation process of the protein of the present invention, the measurement of its molecular weight, its activities on various cells and the presence or absence of its sugar chain moiety. It is to be understood, however, that the examples are for purpose of illustration only and are not intended as a definition of the limits of the invention.

##### Example 1

##### (A) Preparation of the protein, measurement of its molecular weight and determination of its amino acid sequence

(1) To 10 liters of HUOCA-III serum-free culture supernatant was added CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; Dojin Kagaku K.K.) to a final concentration of 0.03%. The thus prepared serum-free culture supernatant was applied to a 40 ml volume of S-Sepharose (Fast Flow, Pharmacia) which has been equilibrated in advance with 10 mM phosphate buffer (pH 7.2) containing 0.15 M NaCl and 0.03% CHAPS, and the contents were adsorbed at a flow rate of 200 ml/hour at 4°C. After washing with the just described buffer solution containing 0.15 M NaCl, the adsorbed contents were eluted by a linear NaCl gradient using two buffers containing 0.15 M NaCl and 2.0 M NaCl at a flow rate of 200 ml/hour

and at a temperature of 4°C. The eluate was checked for its absorbance at 280 nm and collected as fractions of 6.7 ml/tube. Results of the absorbance measurement at 280 nm are shown in Fig. 1.

Each of the thus collected fractions was checked for its activity to enhance the growth of bovine aorta endothelial cells in the following manner. As shown in Fig. 2, the cell growth enhancing activity was found mostly in fractions 12 to 24.

#### Measurement of activity to enhance the growth of bovine aorta endothelial cells

Bovine aorta endothelial cells were suspended in DME (Dulbecco's Modified Eagle's) medium (Flow Laboratories, Inc.) which has been supplemented with 10% fetal calf serum, and the cell suspension was poured in a 24 well multi-dish (Corning Glassworks) with a density of  $5 \times 10^3$  cells/well. On the following day, the medium was replaced by fresh DME medium containing 5% fetal calf serum, and a sample to be tested was added to the fresh medium, followed by 4 days of culturing to measure the number of resulting cells.

(2) The fractions obtained in the above step (1) having high vascular endothelial cell growth-enhancing activities were pooled and diluted with a buffer solution by a factor of 3, and the contents were adsorbed to heparin-Sepharose CL-6B (Pharmacia; bed volume, 4 ml) which has been equilibrated in advance with a buffer solution containing 0.5 M NaCl, at a flow rate of from 0.2 to 0.4 ml/minute and at a temperature of 4°C. After washing with the same buffer solution containing 0.5 M NaCl, the adsorbed contents were eluted by a linear NaCl gradient using two buffers containing 0.5 M NaCl and 2.0 M NaCl at a flow rate of 0.2 ml/min and at a temperature of 4°C. The eluate was checked for its absorbance at 280 nm and collected as fractions of 3 ml/tube. Results of the absorbance measurement at 280 nm are shown in Fig. 3.

Each of the thus collected fractions was checked for its activity to enhance the growth of bovine aorta endothelial cells in the same manner as described above. As shown in fig. 4, the cell growth enhancing activity was found mostly in fractions 23 to 30.

(3) The fractions obtained in the above step (2) having high vascular endothelial cell growth-enhancing activities were pooled and diluted with a buffer solution by a factor of 3, and the contents were adsorbed on to a TSK-heparin 5PW column (7.5 mm in inside diameter and 7.5 cm in length; Tosoh Corp.) which has been equilibrated in advance with a buffer solution containing 0.5 M NaCl. After washing with the same buffer solution containing 0.5 M NaCl, the adsorbed contents were eluted by a linear NaCl gradient using two buffers containing 0.5 M NaCl and 2.0 M NaCl, at a flow rate of 0.5 ml/min and at room temperature. The eluate was checked for its absorbance at 215 nm and collected as fractions of 0.5 ml/tube. Results of the absorbance measurement at 215 nm are shown in Fig. 5.

Each of the thus collected fractions was checked for its activity to enhance the growth of bovine aorta endothelial cells in the same manner as described above. As shown in Fig. 6, the cell growth enhancing activity was found mostly in fractions 30 to 32.

(4) The fractions obtained in the above step (3) having high vascular endothelial cell growth-enhancing activities were pooled and subjected to reverse phase chromatography using a vp-318 column (4.6 mm in inside diameter and 30 mm in length; Senshu Kagaku Co., Ltd.). In the presence of 0.1% trifluoroacetic acid (TFA), a linear gradient elution was carried out by increasing the concentration of acetonitrile from 10% to 60%, at a flow rate of 1.0 ml/min. The eluate was checked for its absorbance at 215 nm and collected as fractions of 10 ml/tube. Results of the absorbance measurement at 215 nm are shown in Fig. 7.

Each of the thus collected fractions was checked for its activity to enhance the growth of bovine aorta endothelial cells in the same manner as described above, with the results shown in Fig. 8. By collecting peak fractions, a highly purified product having high vascular endothelial cell growth-enhancing activity was obtained.

(5) The molecular weight of the highly purified product obtained in the above step (4) was measured by SDS polyacrylamide gel electrophoresis.

The following 6 authentic samples whose molecular weights have been confirmed were used as molecular weight markers, and the electrophoresis was carried out in the same manner as described in the foregoing.



[Molecular weight markers]	
1. Rabbit muscle phosphorylase	(M.W., 97,400 daltons)
2. Bovine serum albumin	(M.W., 66,200 daltons)
3. Ovalbumin	(M.W., 45,000 daltons)
4. Carbonic anhydrase	(M.W., 31,000 daltons)
5. Soybean trypsin inhibitor	(M.W., 21,500 daltons)
6. Lysozyme	(M.W., 14,400 daltons)

The thus obtained electrophoresis pattern is shown in Fig. 9. As is evident from the figure, the highly purified product obtained in the above step (4) has a molecular weight of 72,000 to 80,000 daltons under non-reducing condition, or 79,000 to 85,000 daltons under reducing condition, when measured by SDS polyacrylamide gel electrophoresis. It is evident also that the purified product is a single chain protein.

After the electrophoresis, the gel was cut out at intervals of 2 mm. Each of the thus cut portions was put into a test tube, ground into pieces, mixed with 500  $\mu$ l of a buffer solution 0.03% CHAPS, 20 mmol PB pH 7.2 and then shaken at 4°C for 16 hours. The resulting mixture was centrifuged to recover supernatant fluid which was subsequently dialyzed against a buffer solution 0.03% CHAPS, 20 mmol PB pH 7.2. Contents in the thus dialyzed solution was freeze-dried and then dissolved in 100  $\mu$ l of a buffer solution 0.03% CHAPS, 20 mmol PB pH 7.2 to measure the activity to enhance the growth of bovine aorta endothelial cells in the same manner as described in the foregoing. As shown in Figure 10, the endothelial cell growth-enhancing activity was observed in 72,000-80,000 molecular weight fraction obtained under non-reducing condition.

When the amino acid sequence of the highly purified product was determined in accordance with the procedure described in the foregoing, it was confirmed that the product contained three peptide chains respectively represented by the Sequence ID Nos. 1, 2 and 3.

Also, in order to confirm the addition of sugar chains to the highly purified product, 5  $\mu$ l (250 ng) of the high purity product and 3.2  $\mu$ l of N-glycanase (Genzyme Corp.; 250 units/ml) were added to 30  $\mu$ l of 50 mM Tris-HCl buffer (pH 8.0). After 18 hours of reaction, the resulting mixture was subjected to 0.1% SDS-10% polyacrylamide gel electrophoresis, followed by silver staining. As shown in Fig. 11, the resulting electrophoresis pattern clearly indicated a decrease in the molecular weight of the N-glycanase-treated product due to the separation of sugar chains.

#### (B) Cloning of the DNA and estimation of the amino acid sequence

##### (a) Synthesis of the cDNA

A 5  $\mu$ l portion of the total RNA sample (10  $\mu$ g/ $\mu$ l) which has been prepared from the human ovarian tumor cell line HUOCA-III by the SDS-phenol method was incubated at 70°C for 5 minutes and then cooled down rapidly. After 5 minutes of cooling on an ice bath, to this were added 10  $\mu$ l of a 5 x buffer solution for reverse transcription use (250 mM Tris-HCl/pH 8.3, 375 mM KCl, 15 mM MgCl<sub>2</sub>), 15  $\mu$ l of 2.5 mM dNTP (a mixture of dATP, dCTP, dGTP and dTTP; Takara Shuzo Co., Ltd.), 0.5  $\mu$ l of 1 M DTT (dithiothreitol), 1  $\mu$ l of oligo(dT)<sub>12-18</sub> (Amersham), 2.5  $\mu$ l of a ribonuclease inhibitor (200 U/ $\mu$ l, Takara Shuzo Co., Ltd.), 13  $\mu$ l of distilled water and 3  $\mu$ l of M-MLV reverse transcriptase (200 U/ $\mu$ l, GIBCO-BRL). The thus prepared mixture was incubated at 37°C for 1 hour to effect cDNA synthesis. After removing the proteinous materials from the resulting reaction mixture by phenol treatment, the cDNA of interest was recovered by ethanol precipitation, dissolved in 50  $\mu$ l of distilled water and then stored at -80°C.

##### (b) Amplification of the cDNA which encodes the HUOCA-III-originated novel protein by polymerase chain reaction (PCR)

To 5  $\mu$ l of the cDNA aqueous solution were added 70  $\mu$ l of distilled water, 10  $\mu$ l of a 10 x buffer solution for PCR use (500 mM KCl, 15 mM MgCl<sub>2</sub>, 100 mM Tris-HCl/pH 8.3, 0.01% (w/v) gelatin), 8  $\mu$ l of dNTP (Takara Shuzo Co., Ltd.), 3  $\mu$ l of a 5' primer (5' TCTTTTAGGCACTGACTCCGAACAGGATTCTTTCAC 3', 1  $\mu$ g/ $\mu$ l) and 3  $\mu$ l of a 3' primer (5' GTTGTATTGGTGGATCCTTCAGACACACTTACTTCAG 3'). The thus prepared mixture was incubated at 95°C for 7 minutes, followed by rapid cooling. The thus treated solution was mixed with 1  $\mu$ l

of Ampli Taq DNA polymerase (5 U/ $\mu$ l, Perkin Elmer Cetus), and the surface of the reaction solution was covered with mineral oil (nujol mineral oil manufactured by Perkin Elmer Cetus). Thereafter, PCR was carried out by 30 repetitions of a three step reaction (94°C for 1 minute, 60°C for 2 minutes and 72°C for 3 minutes). After completion of the reaction, mineral oil was removed by chloroform treatment, proteinous materials were removed by phenol treatment and then the PCR product was recovered by ethanol precipitation.

(c) Digestion of the PCR product with BamHI

An 85  $\mu$ l portion of the PCR product was mixed with 10  $\mu$ l of a 10 x buffer solution for BamHI reaction use (1.5 M NaCl, 60 mM Tris-HCl/pH 7.9, 60 mM MgCl<sub>2</sub>) and 5  $\mu$ l of an aqueous solution of BamHI (15 U/ $\mu$ l, Nippon Gene), and the resulting mixture was incubated at 37°C for 1 hour.

(d) Purification of the BamHI-digested PCR product

The PCR product thus digested with BamHI was subjected to 0.7% agarose gel electrophoresis at a constant voltage (100 V). After completion of the electrophoresis, the gel was stained with ethidium bromide to observe DNA bands using a UV transilluminator. A portion of the gel where a DNA band of 2.3 kb was observed was cut out, and the PCR product in the cut portion was purified using Sephaglas Band Prep Kit (Pharmacia).

(e) Digestion of the pUC18 plasmid vector with BamHI

A 2  $\mu$ l portion of pUC18 solution (1  $\mu$ g/ $\mu$ l, Takara Shuzo Co., Ltd.) was mixed with 6.6  $\mu$ l of distilled water, 3  $\mu$ l of the 10 x buffer solution for BamHI reaction use and 1.4  $\mu$ l of BamHI (15 U/ $\mu$ l, Nippon Gene), and the resulting mixture was incubated at 37°C for 1 hour to digest the plasmid. After completion of the reaction, proteinous materials were removed by phenol treatment and the thus digested plasmid fragments were recovered by ethanol precipitation. The thus recovered plasmid fragments were dissolved in 33  $\mu$ l of distilled water and mixed with 4  $\mu$ l of CIP buffer (50 mM Tris-HCl/pH 8.0, 1 mM MgCl<sub>2</sub>) and 3  $\mu$ l of alkaline phosphatase (calf intestine origin, 2,500 U/ml, Toyobo Co., Ltd.). The resulting mixture was incubated at 37°C for 40 minutes and then at 50°C for 20 minutes. After completion of the reaction, the BamHI-digested fragments of the plasmid vector pUC18 were recovered by phenol treatment and subsequent ethanol treatment.

(f) Transformation of *E. coli* JM109 with the PCR product

To 6  $\mu$ l (30  $\mu$ g) of the the BamHI-digested PCR product were added 2  $\mu$ l (200  $\mu$ g) of the pUC18 digest prepared in the above step (e), 2  $\mu$ l of a 10 x ligation buffer solution (10 mM ATP, 200 mM DTT, 100 mM MgCl<sub>2</sub>, 500 mM Tris-HCl/pH 7.9), 9  $\mu$ l of distilled water and 1  $\mu$ l of T4 DNA ligase (500 U/ $\mu$ l, Nippon Gene). After overnight reaction at 16°C, a portion of the resulting reaction solution was added to 100  $\mu$ l of a suspension of *E. coli* JM109 competent cells (Nippon Gene). The thus prepared mixture was allowed to stand still for 20 minutes on an ice bath, heat-treated at 42°C for 45 seconds and then allowed again to stand still on an ice bath for at least 2 minutes. The thus treated mixture was added to 400  $\mu$ l of High-competence broth (Nippon Gene) and stirred on a shaker at 37°C for 60 minutes. To this were added 40  $\mu$ l of 2% X-Gal (5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside) dissolved in diethylformamide and 40  $\mu$ l of 100 mM IPTG (isopropyl- $\beta$ -D-thio-galactopyranoside). The thus prepared mixture was poured on LB plate medium (0.5% yeast extract, 1% Bacto-Trypton, 1.5% agar, 1% NaCl, 50  $\mu$ g/ml ampicillin, pH 7.5) and incubated overnight at 37°C to find white (recombinant) colonies and blue (non-recombinant) colonies grown on the medium. By isolating white colonies, a JM109 transformant into which the cDNA of interest has been inserted was selected.

(g) Preparation of the plasmid

The plasmid-introduced JM109 was cultured overnight at 37°C in 100 ml of LB medium (1% Bacto-Trypton, 0.5% yeast extract, 1% NaCl, pH 7.5). When the cells reached their logarithmic growth phase, they were collected by centrifugation (5 minutes, 5,000 rpm, 0°C) and suspended in 4 ml of P1 buffer solution (100  $\mu$ g/ml RNase A, 50 mM Tris-HCl/pH 8.0, 10 mM EDTA). The resulting cell suspension was mixed with 4 ml of P2 buffer solution (200 mM NaOH, 1% SDS) to carry out an alkali treatment at room temperature for 5 minutes. After the alkali denaturation, the resulting mixture was neutralized by adding 4 ml of P3 buffer solution (2.55 mM Potassium acetate, pH 4.8) and then centrifuged at 15,000 rpm for 30 minutes at 4°C. The thus obtained supernatant fluid was applied to a QIAGEN-MIDI column-pack 100 (DIAGEN) which has been equilibrated in advance with 2 ml of QB buffer solution (750 mM NaCl, 50 mM MOPS [3-(N-morpholino)propanesulfonic acid]/pH

7.0, 15% ethanol). After washing the column twice with 4 ml of QC buffer solution (1 M NaCl, 50 mM MOPS/pH 7.0, 15% ethanol), the plasmid was eluted with 2 ml of QF buffer solution (1.2 M NaCl, 15% ethanol, 50 mM MOPS/pH 8.0). The eluate was mixed with 500  $\mu$ l of isopropanol and centrifuged at room temperature for 30 minutes. Thereafter, the precipitate thus obtained was washed with 70% ethanol and dissolved in 100  $\mu$ l of distilled water.

(h) Determination of the nucleotide sequence by the dideoxy method

A 16  $\mu$ l (3  $\mu$ g) portion of the plasmid solution prepared in the above step (g) was mixed with 2  $\mu$ l of 2 N NaOH and 2  $\mu$ l of 2 mM EDTA, and the mixture was incubated at 37°C for 25 minutes to denature the plasmid. After the alkali denaturation, the resulting solution was mixed with 2  $\mu$ l of 3 M sodium acetate and 100  $\mu$ l of cold ethanol, and ethanol precipitation was effected by maintaining the mixture for 10 minutes at -80°C. The thus precipitated plasmid was recovered by centrifugation, washed with 70% ethanol and then dissolved in 7  $\mu$ l of distilled water. To this were added 1  $\mu$ l of a primer (0.5 pmole) and 2  $\mu$ l of a 5 x buffer solution A (250 mM NaCl, 200 mM Tris-HCl/pH 7.5, 100 mM MgCl<sub>2</sub>). After 2 minutes of incubation at 65°C, the resulting solution was gradually cooled down to 30°C to effect annealing of the denatured plasmid and the primer. To the resulting solution were added 1  $\mu$ l of 0.1 M dithiothreitol, 2  $\mu$ l of a labeling mixture (1.5  $\mu$ M 7-deaza-dGTP, 1.5  $\mu$ M dATP, 1.5  $\mu$ M dTTP), 0.5  $\mu$ l of [ $\alpha$ -<sup>35</sup>S]dCTP (1,000 Ci/mmole, Amersham) and 2  $\mu$ l of Sequenase Ver. 2.0 (1.5 U/ $\mu$ l, United States Biochemical Corporation). After 5 minutes of reaction at 37°C, a 3.5  $\mu$ l portion of the resulting reaction mixture was added to 2.5  $\mu$ l of each of a G solution (80  $\mu$ M 7-deaza-dGTP, 80  $\mu$ M dATP, 80  $\mu$ M dCTP, 80  $\mu$ M dTTP, 8  $\mu$ M ddGTP, 50 mM NaCl), an A solution (80  $\mu$ M 7-deaza-dGTP, 80  $\mu$ M dATP, 80  $\mu$ M dCTP, 80  $\mu$ M dTTP, 8  $\mu$ M ddATP, 50 mM NaCl), a C solution (80  $\mu$ M 7-deaza-dGTP, 80  $\mu$ M dATP, 80  $\mu$ M dCTP, 80  $\mu$ M dTTP, 8  $\mu$ M ddCTP, 50 mM NaCl) and a T solution (80  $\mu$ M 7-deaza-dGTP, 80  $\mu$ M dATP, 80  $\mu$ M dCTP, 80  $\mu$ M dTTP, 8  $\mu$ M ddTTP, 50 mM NaCl). In this instance, each of these solutions was kept at 37°C prior to its use. After 5 minutes of reaction at 37°C, the reaction was terminated by adding 4  $\mu$ l of a reaction termination solution (95% formamide, 0.05% Bromophenol Blue, 20 mM EDTA, 0.05% Xylene Cyanol FF). Thereafter, the reaction mixture was heated at 90°C for 5 minutes, followed by rapid cooling, and a 2.5  $\mu$ l portion of the resulting sample was subjected to electrophoresis. In this case, a composition consisting of 7 M urea, 10% HydroLink™ LONG-RANGER (AT Biochem), 100 mM Tris-HCl, 100 mM boric acid and 2 mM EDTA was made into gel using 0.05% ammonium persulfate and 0.0005% N,N,N',N'-tetramethylethylenediamine (TEMED), and the electrophoresis was carried out at a constant power of 60 W using a TEB buffer (50 mM Tris, 50 mM boric acid, 1 mM EDTA). After completion of the electrophoresis, the gel was dried on a filter paper and subjected to autoradiography to determine the nucleotide sequence of the DNA of interest.

The thus determined DNA sequence is shown in the Sequence ID No. 5, and an amino acid sequence deduced from the DNA sequence is shown in the sequence ID No. 4.

As generally known in this art, the amino acid sequence shown in the Sequence ID No. 4 has a signal peptide. Therefore, the protein of the present invention may be the whole Sequence ID No. 4, a portion of the sequence (for example, the Sequence ID No. 4 except the sequence of a signal peptide), or the portion of the Sequence together with a linker.

The protein of the present invention includes at least an active portion having an activity to enhance the growth of vascular endothelial cells obtainable from a nucleotide sequence or a portion of the nucleotide sequence represented by the Sequence ID No. 5. The DNA corresponding to the signal peptide in the nucleotide sequence represented by the Sequence ID No. 5 may be changed another DNA corresponding to another signal peptide, if necessary, a signal peptide together with a linker DNA sequence may be used in the DNA fragment represented by the Sequence ID No. 5 attached hereto.

#### Example 2 Affinity for concanavalin A

The highly purified product obtained in the step (4) of Example 1 was checked for its affinity for concanavalin A in accordance with the procedure described in the foregoing. As the results, it was confirmed that the purified product was possessed of the affinity for concanavalin A, which is a

In addition, on the basis of the results obtained in Examples 1 and 2, it was confirmed that the high purity product of the step (4) was a single chain glycoprotein.

#### Example 3 New formation of blood vessels

A total of 10 avian eggs, fertilized for 8 days, were used in each test group. A filter (6 mm in diameter) which has been impregnated with a varied amount of the highly purified product (glycoprotein of this invention) ob-

tained in the step (4) of Example 1 was put on the chorio-allantoic membrane of each egg. After 3 days of incubation at 37°C under a moist condition, new formation of blood vessels was observed under a stereoscopic microscope. The judgement was made as positive (+, new formation of blood vessels around the filter) or negative (-, no formation of new blood vessels), and the number of positive eggs in each test group was counted.

As a comparative example, the same experiment was carried out except that the filter was impregnated with physiological saline instead of the purified product. The results are shown in Table 1.

Table 1

Test group	Amount of glycoprotein	Positive effs/Total
1	0 (physiological saline)	0/10
2	1 ng/filter	1/10
3	10 ng/filter	3/10
4	50 ng/filter	5/10
5	100 ng/filter	6/10

It is evident from the above table that the glycoprotein of the present invention is possessed of a function to enhance new formation of blood vessels.

#### Example 4 Growth enhancing effect on human umbilical cord vascular endothelial cells

Human umbilical cord vascular endothelial cells were prepared in the usual way and inoculated into a collagen-coated 24 well multi-dish (Corning Glassworks) with a cell density of  $1 \times 10^4$  cells/well, using MCDB107 medium (Kyokuto Pharmaceutical Industrial Co., Ltd.) supplemented with 20% fetal calf serum. At intervals of 2 days from the next day, the medium was exchanged for a fresh medium containing 5% fetal calf serum and a predetermined amount (see Table 2) of the glycoprotein of the present invention obtained in the step (4) of Example 1. The number of cells was counted on the eighth day, with the results shown in Table 2.

Table 2

Glycoprotein (ng/ml)	Cell count (cells/well)
0	27168
0.3	29460
1.0	30920
3.3	37492
10.0	43072
33.3	54772
100.0	53988
333	46460

As is evident from the above table, the glycoprotein of the present invention is possessed of a function to enhance the growth of human umbilical cord vascular endothelial cells.

#### Example 5 Presence/absence examination of growth enhancing effect on fibroblasts

A primary culture of human dermis fibroblasts prepared from human skin was subcultured, and the eighth subculture was inoculated into a 24 well multi-dish with a cell density of  $5 \times 10^3$  cells/well, using DME medium (Flow Laboratories, Inc.) supplemented with 10% fetal calf serum. At intervals of 2 days from the next day, the medium was exchanged for fresh DME medium containing 0.5% fetal calf serum and 100 ng/ml of the glycoprotein of the present invention obtained in the step (4) of Example 1.

As a comparative example, the same procedure was repeated except that the glycoprotein was eliminated

from the medium or a basic fibroblast growth factor (bFGF) was used in an amount of 1 ng/ml instead of the glycoprotein.

The number of cells was counted on the eighth day, with the results shown in Table 3.

Table 3

Component added	Cell count on 8th day (cells/well)
No addition	28248
Glycoprotein of Example 1	24325
bFGF	42645

As is evident from the above table, bFGF strongly enhances the growth of fibroblasts, but the number of fibroblasts on the eighth day in the case of the addition of the glycoprotein of the present invention obtained in Example 1 is almost the same as that of the case of the control (no addition), thus showing that the inventive glycoprotein hardly has a function to enhance the growth of fibroblasts.

#### Example 6 Presence/absence examination of growth enhancing effect on vascular smooth muscle cells

A primary culture of human smooth muscle cells prepared from an umbilical cord was subcultured, and the sixth subculture was inoculated into a 24 well multi-dish with a cell density of  $5 \times 10^3$  cells/well, using DME medium supplemented with 10% fetal calf serum. At intervals of 2 days from the next day, the medium was exchanged for fresh medium containing 100 ng/ml of the glycoprotein of the present invention obtained in the step (4) of Example 1.

As a comparative example, the same procedure was repeated except that the glycoprotein was eliminated from the medium or a basic fibroblast growth factor (bFGF) was used in an amount of 1 ng/ml instead of the glycoprotein.

The number of cells was counted on the eighth day, with the results shown in Table 4.

Table 4

Component added	Cell count on 8th day (cells/well)
No addition	6192
Glycoprotein of Example 1	7480
bFGF	48962

As is evident from the above table, the number of smooth muscle cells on the eighth day in the case of the addition of the glycoprotein of the present invention obtained in Example 1 is almost the same as that of the case of the control (no addition), thus showing that the inventive glycoprotein has no activity to enhance the growth of human smooth muscle cells.

#### Example 7 Presence/absence examination of growth enhancing effect on hepatocytes

Hepatic parenchymal cells (to be referred to as "hepatocytes" hereinafter) were prepared in accordance with the procedure of Takahashi et al. (*Tissue Culture*, vol.12, No.8, pp.308 - 312, 1986). The thus prepared hepatocytes were suspended in an inoculation medium (WE basal medium supplemented with 5% fetal calf serum and  $10^{-8}$  M dexamethasone) to a cell density of  $5.0 \times 10^4$  cells/0.2 ml, and the resulting hepatocyte suspension was inoculated into a collagen-coated 24 well multi-dish. After 4 hours of the culturing, the medium was replaced by WE basal medium and the glycoprotein of the present invention obtained in Example 1 was added to the fresh medium in a predetermined amount as shown in Table 5. The same process was repeated after additional 16 hours of the culturing. The medium was exchanged again for fresh WE basal medium 40 hours after the commencement of the culturing, and  $^3\text{H}$ -thymidine was added to the fresh medium to carry out 2 hours of pulse-labeling. After completion of the pulse-labeling, the culture supernatant was removed, and the remaining cells were washed with a cold phosphate buffer (PBS), 2% perchlorate and 95% cold ethanol in that order and then dried at room temperature. In this instance, each washing step was repeated three times. The thus dried cells in each well were lysed by adding 0.8 ml of a 1% SDS/0.1 N NaOH solution and maintaining

the mixture at 37°C for at least 1 hour. A 0.5 ml portion of the resulting lysate was pipetted off from each well and put into a scintillation vial. Thereafter, the content in the vial was mixed with 7 ml of a scintillator (OptiFlow, Packard), and the radioactivity was measured using a scintillation counter to examine  $^3\text{H}$ -thymidine uptake.

As a comparative example, the same experiment was carried out except that a mixture of insulin (100 nM/ml) and epidermal growth factor (EGF, 50 ng/ml) was used instead of the glycoprotein of the present invention.

The results are shown in Table 5.

Table 5

Component added	Uptake of $^3\text{H}$ -thymide
<u>Glycoprotein of Example 1</u>	
300 ng/ml	5697 DPM
100 ng/ml	4347 DPM
30 ng/ml	4869 DPM
10 ng/ml	4619 DPM
<u>Insulin + EGF</u>	76815 DPM
(100 nM + 50 ng/ml)	
Control (no addition)	4992 DPM

As is evident from the above table, uptake of  $^3\text{H}$ -thymidine does not occur by the addition of the glycoprotein of the present invention, thus showing that the inventive glycoprotein has no activity to enhance the growth of hepatocytes.

#### Example 8 Presence/absence examination of growth enhancing or inhibiting effect on HeLa cells

HeLa-S3 cells were suspended in MEM medium containing 5% bovine serum to a cell density of  $1 \times 10^5$  cells/ml. The thus prepared HeLa-S3 cell suspension was dispensed in 100  $\mu\text{l}$  portions into wells of a 96 well multi-dish. After 24 hours of culturing, the resulting medium was replaced by fresh MEM medium which has been supplemented with 5% fetal calf serum and a predetermined amount of the glycoprotein obtained in Example 1, and the culturing was continued for additional 48 hours.

Since the presence or absence of the growth inhibiting effect was not able to be judged clearly with the naked eye under a phase-contrast microscope, the judgement was made by staining the cells with Crystal Violet. That is, each well of the dish after the culturing was washed with a phosphate buffer and then filled with a 10% formalin solution for a period of 30 minutes to fix the cells. The thus treated dish was dried after washing it with running water to remove formalin, and the cells in the dish were stained for 15 minutes with a 0.2% Crystal Violet solution containing 2% ethanol. After removing unbound pigment by washing the dish in running water, and subsequently drying the dish, a predetermined amount of 1% sodium dodecyl sulfate solution was added to each well to dissolve the bound pigment. Thereafter, absorbance of the thus dissolved Crystal Violet was measured at a wave length of 540 nm.

As a control, the same culturing step was repeated except that the glycoprotein was not used, and the Crystal Violet staining and absorbance measurement at 540 nm were carried out in the same manner.

The results are shown in Table 6 in which the absorbance of the control at 540 nm is expressed as 1.00.

Table 6

Component added	Ratio of absorbance at 540 nm
<u>Glycoprotein of Example 1</u>	
300 ng/ml	1.02
100 ng/ml	1.01
30 ng/ml	1.01
10 ng/ml	1.02
Control (no addition)	1.00

As shown in the above table, the absorbance at 540 nm hardly changed by the addition of the glycoprotein of the present invention in comparison with the case of the control (no addition), thus confirming that the inventive glycoprotein has no activity to enhance or inhibit the growth of HeLa cells.

#### Example 9 Migration-stimulating activity on vascular endothelial cells and smooth muscle cells

Primary culturing of vascular endothelial cells was carried out by isolating the cells from rabbit cornea capillary vessels in the usual way. The migration-stimulating activity of the cells was measured in accordance with the Boyden's test using Boyden's chamber. That is, DME medium supplemented with 10% fetal calf serum and a predetermined amount of the glycoprotein obtained in Example 1 was put into the lower compartment of the Boyden's chamber, and another DME medium supplemented with 10% fetal calf serum and  $2 \times 10^4$ /ml of vascular endothelial cells was put into the upper compartment of the chamber. Thereafter, culturing was carried out at 37°C for 4 hours.

A similar test was carried out using primary-cultured smooth muscle cells which have been isolated from rat pulmonary artery

After the culturing, the thus treated cells were stained with Diff-Quick solution, and the number of migrated cells per visual field was counted under a microscope, with the results shown in Table 7.

Table 7

Glycoprotein	The number of migrated cells	
	Vascular endothelial cells	Smooth muscle cells
300 ng/ml	268	0
100 ng/ml	50	0
30 ng/ml	37	0

As is evident from the above table, the glycoprotein of the present invention shows migration-stimulating activity on vascular endothelial cells but not on smooth muscle cells.

Thus, it is apparent that there has been provided, in accordance with the present invention, a novel protein of human origin, as well as a process for the production thereof. Since the protein of the present invention enhances the growth of vascular endothelial cells but does not activate the growth of smooth muscle cells, fibroblasts and hepatocytes and also does not enhance or inhibit the growth of HeLa cells, it can enhance the growth of vascular endothelial cells selectively and therefore can enhance new formation of blood vessels smoothly without causing secondary reactions. Because of such excellent properties, especially its activity to enhance new formation of blood vessels, the protein of the present invention can be applied to a healing enhancer of wound, burn injury, decubitus, postoperative tissue damage or the like or as a drug for the treatment of cardiac angiopathy, as well as its application to artificial organs such as artificial blood vessel, artificial skin and the like. It also can be applied to diagnostic and therapeutic drugs of malignant tumor, retinopathy, chronic rheumatoid arthritis and the like.

In addition, the protein of the present invention can be obtained with a high productivity and a high purity in comparison with the prior art physiologically active factors.

SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

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- (C) CITY: TOKYO
- (E) COUNTRY: JAPAN
- (F) POSTAL CODE (ZIP): 151

15 (ii) TITLE OF INVENTION: Novel protein of human origin and its  
production process

20 (iii) NUMBER OF SEQUENCES: 7

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- 25 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:

30 APPLICATION NUMBER: EP 92 403 199.0

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: JP 3-337999
- (B) FILING DATE: 28-NOV-1991

35

(2) INFORMATION FOR SEQ ID NO: 1:

40 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

50

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- 55 (G) CELL TYPE: Ovarian
- (H) CELL LINE: HUOCA II / HUOCA III



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

5 Arg Asn Thr Ile His Glu Phe  
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear  
15  
(ii) MOLECULE TYPE: peptide  
(iii) HYPOTHETICAL: NO  
20  
(v) FRAGMENT TYPE: internal  
(vi) ORIGINAL SOURCE:  
25 (A) ORGANISM: Homo sapiens  
(G) CELL TYPE: Ovarian  
(H) CELL LINE: HUOCA II / HUOCA III

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Glu Phe Gly His Glu Phe Asp Leu Tyr Glu  
1 5 10

35 (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:  
40 (A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear  
45  
(ii) MOLECULE TYPE: peptide  
(iii) HYPOTHETICAL: NO  
(v) FRAGMENT TYPE: C-terminal  
50  
(vi) ORIGINAL SOURCE:  
55 (A) ORGANISM: Homo sapiens  
(G) CELL TYPE: Ovarian  
(H) CELL LINE: HUOCA II / HUOCA III

## (ix) FEATURE:

(A) NAME/KEY: Modified-site  
 (B) LOCATION: 3  
 (D) OTHER INFORMATION: /label= Xaa  
 /note= "unidentified amino acid residue"

## (ix) FEATURE:

(A) NAME/KEY: Modified-site  
 (B) LOCATION: 10  
 (D) OTHER INFORMATION: /label= Xaa  
 /note= "unidentified amino acid residue"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Glu Ser Xaa Val Leu Thr Ala Arg Gln Xaa Phe Pro Ser Arg Asp Leu  
 1 5 10 15

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 728 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: YES

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens  
 (G) CELL TYPE: ovarian  
 (H) CELL LINE: HUOCA II / HUOCA III

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Trp Val Thr Lys Leu Leu Pro Ala Leu Leu Leu Gln His Val Leu  
 1 5 10 15  
 Leu His Leu Leu Leu Leu Pro Ile Ala Ile Pro Tyr Ala Glu Gly Gln  
 20 25 30  
 Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser Ala Lys Thr  
 35 40 45

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	Thr	Leu	Ile	Lys	Ile	Asp	Pro	Ala	Leu	Lys	Ile	Lys	Thr	Lys	Lys	Val	
	50						55					60					
5	Asn	Thr	Ala	Asp	Gln	Cys	Ala	Asn	Arg	Cys	Thr	Arg	Asn	Lys	Gly	Leu	
	65				70					75					80		
	Pro	Phe	Thr	Cys	Lys	Ala	Phe	Val	Phe	Asp	Lys	Ala	Arg	Lys	Gln	Cys	
				85					90					95			
10	Leu	Trp	Phe	Pro	Phe	Asn	Ser	Met	Ser	Ser	Gly	Val	Lys	Lys	Glu	Phe	
				100					105					110			
	Gly	His	Glu	Phe	Asp	Leu	Tyr	Glu	Asn	Lys	Asp	Tyr	Ile	Arg	Asn	Cys	
15			115				120						125				
	Ile	Ile	Gly	Lys	Gly	Arg	Ser	Tyr	Lys	Gly	Thr	Val	Ser	Ile	Thr	Lys	
			130				135					140					
20	Ser	Gly	Ile	Lys	Cys	Gln	Pro	Trp	Ser	Ser	Met	Ile	Pro	His	Glu	His	
	145				150					155					160		
	Ser	Phe	Leu	Pro	Ser	Ser	Tyr	Arg	Gly	Lys	Asp	Leu	Gln	Glu	Asn	Tyr	
				165					170					175			
25	Cys	Arg	Asn	Pro	Arg	Gly	Glu	Glu	Gly	Gly	Pro	Trp	Cys	Phe	Thr	Ser	
				180					185					190			
	Asn	Pro	Glu	Val	Arg	Tyr	Glu	Val	Cys	Asp	Ile	Pro	Gln	Cys	Ser	Glu	
30			195				200						205				
	Val	Glu	Cys	Met	Thr	Cys	Asn	Gly	Glu	Ser	Tyr	Arg	Gly	Leu	Met	Asp	
			210				215						220				
	His	Thr	Glu	Ser	Gly	Lys	Ile	Cys	Gln	Arg	Trp	Asp	His	Gln	Thr	Pro	
35	225				230					235					240		
	His	Arg	His	Lys	Phe	Leu	Pro	Glu	Arg	Tyr	Pro	Asp	Lys	Gly	Phe	Asp	
				245					250					255			
40	Asp	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Gln	Pro	Arg	Pro	Trp	Cys	Tyr	
			260						265					270			
	Thr	Leu	Asp	Pro	His	Thr	Arg	Trp	Glu	Tyr	Cys	Ala	Ile	Lys	Thr	Cys	
45			275				280						285				
	Ala	Asp	Asn	Thr	Met	Asn	Asp	Thr	Asp	Val	Pro	Leu	Glu	Thr	Thr	Glu	
			290				295						300				
	Cys	Ile	Gln	Gly	Gln	Gly	Glu	Gly	Tyr	Arg	Gly	Thr	Val	Asn	Thr	Ile	
50	305				310					315					320		
	Trp	Asn	Gly	Ile	Pro	Cys	Gln	Arg	Trp	Asp	Ser	Gln	Tyr	Pro	His	Glu	
				325					330					335			
55	His	Asp	Met	Thr	Pro	Glu	Asn	Phe	Lys	Cys	Lys	Asp	Leu	Arg	Glu	Asn	
			340						345					350			

Tyr Cys Arg Asn Pro Asp Gly Ser Glu Ser Pro Trp Cys Phe Thr Thr  
 355 360 365  
 5 Asp Pro Asn Ile Arg Val Gly Tyr Cys Ser Gln Ile Pro Asn Cys Asp  
 370 375 380  
 Met Ser His Gly Gln Asp Cys Tyr Arg Gly Asn Gly Lys Asn Tyr Met  
 385 390 395 400  
 10 Gly Asn Leu Ser Gln Thr Arg Ser Gly Leu Thr Cys Ser Met Trp Asp  
 405 410 415  
 Lys Asn Met Glu Asp Leu His Arg His Ile Phe Trp Glu Pro Asp Ala  
 15 420 425 430  
 Ser Lys Leu Asn Glu Asn Tyr Cys Arg Asn Pro Asp Asp Ala His  
 435 440 445  
 20 Gly Pro Trp Cys Tyr Thr Gly Asn Pro Leu Ile Pro Trp Asp Tyr Cys  
 450 455 460  
 Pro Ile Ser Arg Cys Glu Gly Asp Thr Thr Pro Thr Ile Val Asn Leu  
 465 470 475 480  
 25 Asp His Pro Val Ile Ser Cys Ala Lys Thr Lys Gln Leu Arg Val Val  
 485 490 495  
 Asn Gly Ile Pro Thr Arg Thr Asn Ile Gly Trp Met Val Ser Leu Arg  
 30 500 505 510  
 Tyr Arg Asn Lys His Ile Cys Gly Gly Ser Leu Ile Lys Glu Ser Trp  
 515 520 525  
 Val Leu Thr Ala Arg Gln Cys Phe Pro Ser Arg Asp Leu Lys Asp Tyr  
 35 530 535 540  
 Glu Ala Trp Leu Gly Ile His Asp Val His Gly Arg Gly Asp Glu Lys  
 545 550 555 560  
 40 Cys Lys Gln Val Leu Asn Val Ser Gln Leu Val Tyr Gly Pro Glu Gly  
 565 570 575  
 Ser Asp Leu Val Leu Met Lys Leu Ala Arg Pro Ala Val Leu Asp Asp  
 580 585 590  
 45 Phe Val Ser Thr Ile Asp Leu Pro Asn Tyr Gly Cys Thr Ile Pro Glu  
 595 600 605  
 Lys Thr Ser Cys Ser Val Tyr Gly Trp Gly Tyr Thr Gly Leu Ile Asn  
 50 610 615 620  
 Tyr Asp Gly Leu Leu Arg Val Ala His Leu Tyr Ile Met Gly Asn Glu  
 625 630 635 640  
 55 Lys Cys Ser Gln His His Arg Gly Lys Val Thr Leu Asn Glu Ser Glu  
 645 650 655

5 Ile Cys Ala Gly Ala Glu Lys Ile Gly Ser Gly Pro Cys Glu Gly Asp  
                     660                    665                    670  
 Tyr Gly Gly Pro Leu Val Cys Glu Gln His Lys Met Arg Met Val Leu  
                     675                    680                    685  
 10 Gly Val Ile Val Pro Gly Arg Gly Cys Ala Ile Pro Asn Arg Pro Gly  
                     690                    695                    700  
 Ile Phe Val Arg Val Ala Tyr Tyr Ala Lys Trp Ile His Lys Ile Ile  
                     705                    710                    715                    720  
 15 Leu Thr Tyr Lys Val Pro Gln Ser  
                     725

## (2) INFORMATION FOR SEQ ID NO: 5:

20

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2187 base pairs

(B) TYPE: nucleic acid

25

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30

(iii) HYPOTHETICAL: YES

(iii) ANTI-SENSE: NO

35

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

40

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50

55

ATGTGGGTGA CCAAACCTCT GCCAGCCCTG CTGCTGCAGC ATGTCCTCCT GCATCTCCTC 60  
 CTGCTCCCCA TCGCCATCCC CTATGCAGAG GGACAAAGGA AAAGAAGAAA TACAATTCAT 120  
 GAATTCAAAA AATCAGCAAA GACTACCCTA ATCAAAATAG ATCCAGCACT GAAQATAAAA 180  
 ACCAAAAAAG TGAATACTGC AGACCAATGT GCTAATAGAT GTACTAGGAA TAAAGGACTT 240  
 CCATTCACTT GCAAGGCTTT TGTTTTGTGAT AAAGCAAGAA AACAATGCCT CTGGTTCCCC 300  
 TTCAATAGCA TGTCAAGTGG ACTGAAAAAA GAATTTGGCC ATGAATTTGA CCTCTATGAA 360  
 AACAAAGACT ACATTAGAAA CTGCATCATT GGTAAAGGAC GCAGCTACAA GGGAACAGTA 420  
 TCTATCACTA AGAGTGGCAT CAAATGTGAG CCCTGGAGTT CCATGATACC ACACGAACAC 480  
 AGCTTTTTCG CTTGAGCTA TCGGGGTAAA GACCTACAGG AAAACTACTG TCGAAATCCT 540  
 CGAGGGGAAG AAGGGGGACC CTGGTGTTC ACAAGCAATC CAGAGGTACG CTACGAAGTC 600  
 TGTGACATTC CTCAGTGTTC AGAAGTTGAA TGCATGACCT GCAATGGGGA GAGTTATCGA 660  
 GGTCTCATGG ATCATACAGA ATCAGGCAAG ATTTGTCAGC GCTGGGATCA TCAGACACCA 720  
 CACCGGCACA AATTCTTGCC TGAAAGATAT CCGACAAGG GCTTTGATGA TAATTATTGC 780  
 CGCAATCCCG ATGGCCAGCC GAGGCCATGG TGCTATACTC TTGACCCTCA CACCCGCTGG 840  
 GAGTACTGTG CAATTAAAC ATGCGCTGAC AATACTATGA ATGACACTGA TGTTCCTTTG 900  
 GAAACAACTG AATGCATCCA AGGTCAAGGA GAAGGCTACA GGGGCACTGT CAATACCATT 960  
 TGGAATGGAA TTCCATGTCA GCGTTGGGAT TCTCAGTATC CTCACGAGCA TGACATGACT 1020

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5  CCTGAAAATT TCAAGTGCAA GGACCTACGA GAAAATTACT GCCGAAATCC AGATGGGTCT 1080
   GAATCACCCT GGTGTTTTAC CACTGATCCA AACATCCGAG TTGGCTACTG CTCCCAAATT 1140
   CCAAACGTG ATATGTCACA TGGACAAGAT TGTATCGTG GGAATGGCAA AAATTATATG 1200
   GGCAACTTAT CCCAAACAAG ATCTGGACTA ACATGTTCAA TGTGGGACAA GAACATGGAA 1260
   GACTTACATC GTCATATCTT CTGGGAACCA GATGCAAGTA AGCTGAATGA GAATTACTGC 1320
10  CGAAATCCAG ATGATGATGC TCATGGACCC TGGTGCTACA CGGGAAATCC ACTCATTCTT 1380
   TGGGATTATT GCCCTATTTT TCGTTGTGAA GGTGATACCA CACCTACAAT AGTCAATTTA 1440
   GACCATCCCG TAATATCTTG TGCCAAAACG AAACAATTGC GAGITGTAAA TGGGATTCCA 1500
   ACACGAACAA ACATAGGATG GATGGTTAGT TTGAGATACA GAAATAAACA TATCTGCGGA 1560
   GGATCATTGA TAAAGGAGAG TTGGGTTCTT ACTGCACGAC AGTGTTTCCC TTCTCGAGAC 1620
15  TTGAAAGATT ATGAAGCTTG GCTTGGAATT CATGATGTCC ACGGAAGAGG AGATGAGAAA 1680
   TGCAAACAGG TTCTCAATGT TTCCCAGCTG GTATATGGCC CTGAAGGATC AGATCTGGTT 1740
   TTAATGAAGC TTGCCAGGCC TGCTGTCCTG GATGATTTTG TTAGTACGAT TGATTTACCT 1800
   AATTATGGAT GCACAATTCC TGAAAAGACC AGTTGCAGTG TTTATGGCTG GGGCTACACT 1860
20  GGATTGATCA ACTATGATGG CCTATTACGA GTGGCACATC TCTATATAAT GGGAAATGAG 1920
   AAATGCAGCC AGCATCATCG AGGGAAGGTG ACTCTGAATG AGTCTGAAAT ATGTGCTGGG 1980
   GCTGAAAAGA TTGGATCAGG ACCATGTGAG GGGGATTATG GTGGCCCACT TGTTGTGAG 2040
   CAACATAAAA TGAGAATGGT TCTTGGTGTC ATTGTTCTCG GTCGTGGATG TGCCATTCCA 2100
   AATCGTCTG GTATTTTTGT CCGAGTAGCA TATTATGCAA AATGGATACA CAAAATTATT 2160
25  TTAACATATA AGGTACCACA GTCATAG 2187

```

## (2) INFORMATION FOR SEQ ID NO: 6:

## 30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2576 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

40 (iii) HYPOTHETICAL: YES

(iii) ANTI-SENSE: NO

## (ix) FEATURE:

45 (A) NAME/KEY: CDS

(B) LOCATION: join(102..2285, 2289..2294, 2298..2336, 2340  
..2384, 2388..2480, 2484..2507, 2514..2522, 2526  
..2570)

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGGCUCAGAG CCGACUGGCU CUUUUAGGCA CUGACUCCGA ACAGGAUUCU UUCACCCAGG 60

55

5	CAUCUCCUCC AGAGGGAUCC GCCAGCCCGU CCAGCAGCAC C AUG UGG GUG ACC	113
	Met Trp Val Thr	
	1	
10	AAA CUC CUG CCA GCC CUG CUG CUG CAG CAU GUC CUC CUG CAU CUC CUC	161
	Lys Leu Leu Pro Ala Leu Leu Leu Gln His Val Leu Leu His Leu Leu	
	5 10 15 20	
15	CUG CUC CCC AUC GCC AUC CCC UAU GCA GAG GGA CAA AGG AAA AGA AGA	209
	Leu Leu Pro Ile Ala Ile Pro Tyr Ala Glu Gly Gln Arg Lys Arg Arg	
	25 30 35	
20	AAU ACA AUU CAU GAA UUC AAA AAA UCA GCA AAG ACU ACC CUA AUC AAA	257
	Asn Thr Ile His Glu Phe Lys Lys Ser Ala Lys Thr Thr Leu Ile Lys	
	40 45 50	
25	AUA GAU CCA GCA CUG AAG AUA AAA ACC AAA AAA GUG AAU ACU GCA GAC	305
	Ile Asp Pro Ala Leu Lys Ile Lys Thr Lys Lys Val Asn Thr Ala Asp	
	55 60 65	
30	CAA UGU GCU AAU AGA UGU ACU AGG AAU AAA GGA CUU CCA UUC ACU UGC	353
	Gln Cys Ala Asn Arg Cys Thr Arg Asn Lys Gly Leu Pro Phe Thr Cys	
	70 75 80	
35	AAG GCU UUU GUU UUU GAU AAA GCA AGA AAA CAA UGC CUC UGG UUC CCC	401
	Lys Ala Phe Val Phe Asp Lys Ala Arg Lys Gln Cys Leu Trp Phe Pro	
	85 90 95 100	
40	UUC AAU AGC AUG UCA AGU GGA GUG AAA AAA GAA UUU GGC CAU GAA UUU	449
	Phe Asn Ser Met Ser Ser Gly Val Lys Lys Glu Phe Gly His Glu Phe	
	105 110 115	
45	GAC CUC UAU GAA AAC AAA GAC UAC AUU AGA AAC UGC AUC AUU GGU AAA	497
	Asp Leu Tyr Glu Asn Lys Asp Tyr Ile Arg Asn Cys Ile Ile Gly Lys	
	120 125 130	
50	GGA CGC AGC UAC AAG GGA ACA GUA UCU AUC ACU AAG AGU GGC AUC AAA	545
	Gly Arg Ser Tyr Lys Gly Thr Val Ser Ile Thr Lys Ser Gly Ile Lys	
	135 140 145	
55	UGU CAG CCC UGG AGU UCC AUG AUA CCA CAC GAA CAC AGC UUU UUG CCU	593
	Cys Gln Pro Trp Ser Ser Met Ile Pro His Glu His Ser Phe Leu Pro	
	150 155 160	

5	UCG AGC UAU CGG GGU AAA GAC CUA CAG GAA AAC UAC UGU CGA AAU CCU Ser Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr Cys Arg Asn Pro 165 170 175 180	641
10	CGA GGG GAA GAA GGG GGA CCC UGG UGU UUC ACA AGC AAU CCA GAG GUA Arg Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser Asn Pro Glu Val 185 190 195	689
15	CGC UAC GAA GUC UGU GAC AUU CCU CAG UGU UCA GAA GUU GAA UGC AUG Arg Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu Val Glu Cys Met 200 205 210	737
20	ACC UGC AAU GGG GAG AGU UAU CGA GGU CUC AUG GAU CAU ACA GAA UCA Thr Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp His Thr Glu Ser 215 220 225	785
25	GGC AAG AUU UGU CAG CGC UGG GAU CAU CAG ACA CCA CAC CGG CAC AAA Gly Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro His Arg His Lys 230 235 240	833
30	UUC UUG CCU GAA AGA UAU CCC GAC AAG GGC UUU GAU GAU AAU UAU UGC Phe Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe Asp Asp Asn Tyr Cys 245 250 255 260	881
35	CGC AAU CCC GAU GGC CAG CCG AGG CCA UGG UGC UAU ACU CUU GAC CCU Arg Asn Pro Asp Gly Gln Pro Arg Pro Trp Cys Tyr Thr Leu Asp Pro 265 270 275	929
40	CAC ACC CGC UGG GAG UAC UGU GCA AUU AAA ACA UGC GCU GAC AAU ACU His Thr Arg Trp Glu Tyr Cys Ala Ile Lys Thr Cys Ala Asp Asn Thr 280 285 290	977
45	AUG AAU GAC ACU GAU GUU CCU UUG GAA ACA ACU GAA UGC AUC CAA GGU Met Asn Asp Thr Asp Val Pro Leu Glu Thr Thr Glu Cys Ile Gln Gly 295 300 305	1025
50	CAA GGA GAA GGC UAC AGG GGC ACU GUC AAU ACC AUU UGG AAU GGA AUU Gln Gly Glu Gly Tyr Arg Gly Thr Val Asn Thr Ile Trp Asn Gly Ile 310 315 320	1073
55	CCA UGU CAG CGU UGG GAU UCU CAG UAU CCU CAC GAG CAU GAC AUG ACU Pro Cys Gln Arg Trp Asp Ser Gln Tyr Pro His Glu His Asp Met Thr 325 330 335 340	1121



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5	CCU GAA AAU UUC AAG UGC AAG GAC CUA CGA GAA AAU UAC UGC CGA AAU Pro Glu Asn Phe Lys Cys Lys Asp Leu Arg Glu Asn Tyr Cys Arg Asn 345 350 355	1169
10	CCA GAU GGG UCU GAA UCA CCC UGG UGU UUU ACC ACU GAU CCA AAC AUC Pro Asp Gly Ser Glu Ser Pro Trp Cys Phe Thr Thr Asp Pro Asn Ile 360 365 370	1217
15	CGA GUU GGC UAC UGC UCC CAA AUU CCA AAC UGU GAU AUG UCA CAU GGA Arg Val Gly Tyr Cys Ser Gln Ile Pro Asn Cys Asp Met Ser His Gly 375 380 385	1265
20	CAA GAU UGU UAU CGU GGG AAU GGC AAA AAU UAU AUG GGC AAC UUA UCC Gln Asp Cys Tyr Arg Gly Asn Gly Lys Asn Tyr Met Gly Asn Leu Ser 390 395 400	1313
25	CAA ACA AGA UCU GGA CUA ACA UGU UCA AUG UGG GAC AAG AAC AUG GAA Gln Thr Arg Ser Gly Leu Thr Cys Ser Met Trp Asp Lys Asn Met Glu 405 410 415 420	1361
30	GAC UUA CAU CGU CAU AUC UUC UGG GAA CCA GAU GCA AGU AAG CUG AAU Asp Leu His Arg His Ile Phe Trp Glu Pro Asp Ala Ser Lys Leu Asn 425 430 435	1409
35	GAG AAU UAC UGC CGA AAU CCA GAU GAU GAU GCU CAU GGA CCC UGG UGC Glu Asn Tyr Cys Arg Asn Pro Asp Asp Ala His Gly Pro Trp Cys 440 445 450	1457
40	UGU GAA GGU GAU ACC ACA CCU ACA AUA GUC AAU UUA GAC CAU CCC GUA Cys Glu Gly Asp Thr Thr Pro Thr Ile Val Asn Leu Asp His Pro Val 470 475 480	1553
45	AUA UCU UGU GCC AAA ACG AAA CAA UUG CGA GUU GUA AAU GGG AUU CCA Ile Ser Cys Ala Lys Thr Lys Gln Leu Arg Val Val Asn Gly Ile Pro 485 490 495 500	1601
50	ACA CGA ACA AAC AUA GGA UGG AUG GUU AGU UUG AGA UAC AGA AAU AAA Thr Arg Thr Asn Ile Gly Trp Met Val Ser Leu Arg Tyr Arg Asn Lys 505 510 515	1649

65

5	CAU AUC UGC GGA GGA UCA UUG AUA AAG GAG AGU UGG GUU CUU ACU GCA His Ile Cys Gly Gly Ser Leu Ile Lys Glu Ser Trp Val Leu Thr Ala 520 525 530	1697
10	CGA CAG UGU UUC CCU UCU CGA GAC UUG AAA GAU UAU GAA GCU UGG CUU Arg Gln Cys Phe Pro Ser Arg Asp Leu Lys Asp Tyr Glu Ala Trp Leu 535 540 545	1745
15	GGA AUU CAU GAU GUC CAC GGA AGA GGA GAU GAG AAA UGC AAA CAG GUU Gly Ile His Asp Val His Gly Arg Gly Asp Glu Lys Cys Lys Gln Val 550 555 560	1793
20	CUC AAU GUU UCC CAG CUG GUA UAU GGC CCU GAA GGA UCA GAU CUG GUU Leu Asn Val Ser Gln Leu Val Tyr Gly Pro Glu Gly Ser Asp Leu Val 565 570 575 580	1841
25	UUA AUG AAG CUU GCC AGG CCU GCU GUC CUG GAU GAU UUU GUU ACU ACG Leu Met Lys Leu Ala Arg Pro Ala Val Leu Asp Asp Phe Val Ser Thr 585 590 595	1889
30	AUU GAU UUA CCU AAU UAU GGA UGC ACA AUU CCU GAA AAG ACC AGU UGC Ile Asp Leu Pro Asn Tyr Gly Cys Thr Ile Pro Glu Lys Thr Ser Cys 600 605 610	1937
35	AGU GUU UAU GGC UGG GGC UAC ACU GGA UUG AUC AAC UAU GAU GGC CUA Ser Val Tyr Gly Trp Gly Tyr Thr Gly Leu Ile Asn Tyr Asp Gly Leu 615 620 625	1985
40	UUA CGA GUG GCA CAU CUC UAU AUA AUG GGA AAU GAG AAA UGC AGC CAG Leu Arg Val Ala His Leu Tyr Ile Met Gly Asn Glu Lys Cys Ser Gln 630 635 640	2033
45	CAU CAU CGA GGG AAG GUG ACU CUG AAU GAG UCU GAA AUA UGU GCU GGG His His Arg Gly Lys Val Thr Leu Asn Glu Ser Glu Ile Cys Ala Gly 645 650 655 660	2081
50	GCU GAA AAG AUU GGA UCA CGA CCA UGU GAG GGG GAU UAU GGU GGC CCA Ala Glu Lys Ile Gly Ser Gly Pro Cys Glu Gly Asp Tyr Gly Gly Pro 665 670 675	2129
55	CUU GUU UGU GAG CAA CAU AAA AUG AGA AUG GUU CUU GGU GUC AUU GUU Leu Val Cys Glu Gln His Lys Met Arg Met Val Leu Gly Val Ile Val 680 685 690	2177

5 CCU GGU CGU GGA UGU GCC AUU CCA AAU CGU CCU GGU AUU UUU GUC CGA 2225  
Pro Gly Arg Gly Cys Ala Ile Pro Asn Arg Pro Gly Ile Phe Val Arg  
695 700 705

10 GUA GCA UAU UAU GCA AAA UGG AUA CAC AAA AUU AUU UUA ACA UAU AAG 2273  
Val Ala Tyr Tyr Ala Lys Trp Ile His Lys Ile Ile Leu Thr Tyr Lys  
710 715 720

15 GUA CCA CAG UCA UAG CUG AAG UAA GUG UGU CUG AAG CAC CCA CCA AUA 2321  
Val Pro Gln Ser Leu Lys Val Cys Leu Lys His Pro Pro Ile  
725 730 735

20 CAA CUG UCU UUU ACA UGA AGA UUU CAG AGA AUG UGG AAU UUA AAA UGU 2369  
Gln Leu Ser Phe Thr Arg Phe Gln Arg Met Trp Asn Leu Lys Cys  
740 745 750

25 CAC UUA CAA CAA UCC UAA GAC AAC UAC UGG AGA GUC AUG UUU GUU GAA 2417  
His Leu Gln Gln Ser Asp Asn Tyr Trp Arg Val Met Phe Val Glu  
755 760 765

30 AUU CUC AUU AAU GUU UAU GGG UGU UUU CUG UUG UUU UGU UUG UCA GUG 2465  
Ile Leu Ile Asn Val Tyr Gly Cys Phe Leu Leu Phe Cys Leu Ser Val  
770 775 780

35 UUA UUU UGU CAA UGU UGA AGU GAA UUA AGG UAC AUG CAA GUG 2507  
Leu Phe Cys Gln Cys Ser Glu Leu Arg Tyr Met Gln Val  
785 790 795

40 UAAUAA CAU AUC UCC UGA AGA UAC UUG AAU GGA UUA AAA AAA CAC ACA 2555  
His Ile Ser Arg Tyr Leu Asn Gly Leu Lys Lys His Thr  
800 805 810

45 GGU AUA UUU GCU GGA UGAUAA 2576  
Gly Ile Phe Ala Gly  
815

45 (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 815 amino acids

50 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

55

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

5 Met Trp Val Thr Lys Leu Leu Pro Ala Leu Leu Leu Gln His Val Leu  
 1 5 10 15  
 Leu His Leu Leu Leu Leu Pro Ile Ala Ile Pro Tyr Ala Glu Gly Gln  
 20 25 30  
 10 Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser Ala Lys Thr  
 35 40 45  
 Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile Lys Thr Lys Lys Val  
 50 55 60  
 15 Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr Arg Asn Lys Gly Leu  
 65 70 75 80  
 Pro Phe Thr Cys Lys Ala Phe Val Phe Asp Lys Ala Arg Lys Gln Cys  
 85 90 95  
 20 Leu Trp Phe Pro Phe Asn Ser Met Ser Ser Gly Val Lys Lys Glu Phe  
 100 105 110  
 25 Gly His Glu Phe Asp Leu Tyr Glu Asn Lys Asp Tyr Ile Arg Asn Cys  
 115 120 125  
 Ile Ile Gly Lys Gly Arg Ser Tyr Lys Gly Thr Val Ser Ile Thr Lys  
 130 135 140  
 30 Ser Gly Ile Lys Cys Gln Pro Trp Ser Ser Met Ile Pro His Glu His  
 145 150 155 160  
 Ser Phe Leu Pro Ser Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr  
 165 170 175  
 35 Cys Arg Asn Pro Arg Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser  
 180 185 190  
 40 Asn Pro Glu Val Arg Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu  
 195 200 205  
 Val Glu Cys Met Thr Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp  
 210 215 220  
 45 His Thr Glu Ser Gly Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro  
 225 230 235 240  
 His Arg His Lys Phe Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe Asp  
 245 250 255  
 50 Asp Asn Tyr Cys Arg Asn Pro Asp Gly Gln Pro Arg Pro Trp Cys Tyr  
 260 265 270  
 65 Thr Leu Asp Pro His Thr Arg Trp Glu Tyr Cys Ala Ile Lys Thr Cys  
 275 280 285

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	Ala	Asp	Asn	Thr	Met	Asn	Asp	Thr	Asp	Val	Pro	Leu	Glu	Thr	Thr	Glu	
	290						295					300					
5	Cys	Ile	Gln	Gly	Gln	Gly	Glu	Gly	Tyr	Arg	Gly	Thr	Val	Asn	Thr	Ile	
	305					310					315					320	
	Trp	Asn	Gly	Ile	Pro	Cys	Gln	Arg	Trp	Asp	Ser	Gln	Tyr	Pro	His	Glu	
					325					330					335		
10	His	Asp	Met	Thr	Pro	Glu	Asn	Phe	Lys	Cys	Lys	Asp	Leu	Arg	Glu	Asn	
				340					345						350		
	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Ser	Glu	Ser	Pro	Trp	Cys	Phe	Thr	Thr	
15			355					360					365				
	Asp	Pro	Asn	Ile	Arg	Val	Gly	Tyr	Cys	Ser	Gln	Ile	Pro	Asn	Cys	Asp	
	370						375						380				
20	Met	Ser	His	Gly	Gln	Asp	Cys	Tyr	Arg	Gly	Asn	Gly	Lys	Asn	Tyr	Met	
	385					390					395					400	
	Gly	Asn	Leu	Ser	Gln	Thr	Arg	Ser	Gly	Leu	Thr	Cys	Ser	Met	Trp	Asp	
					405					410						415	
25	Lys	Asn	Met	Glu	Asp	Leu	His	Arg	His	Ile	Phe	Trp	Glu	Pro	Asp	Ala	
				420					425						430		
	Ser	Lys	Leu	Asn	Glu	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Asp	Asp	Ala	His	
30			435					440					445				
	Gly	Pro	Trp	Cys	Tyr	Thr	Gly	Asn	Pro	Leu	Ile	Pro	Trp	Asp	Tyr	Cys	
	450						455						460				
	Pro	Ile	Ser	Arg	Cys	Glu	Gly	Asp	Thr	Thr	Pro	Thr	Ile	Val	Asn	Leu	
35	465					470					475					480	
	Asp	His	Pro	Val	Ile	Ser	Cys	Ala	Lys	Thr	Lys	Gln	Leu	Arg	Val	Val	
					485					490					495		
40	Asn	Gly	Ile	Pro	Thr	Arg	Thr	Asn	Ile	Gly	Trp	Met	Val	Ser	Leu	Arg	
				500					505						510		
	Tyr	Arg	Asn	Lys	His	Ile	Cys	Gly	Gly	Ser	Leu	Ile	Lys	Glu	Ser	Trp	
45			515					520					525				
	Val	Leu	Thr	Ala	Arg	Gln	Cys	Phe	Pro	Ser	Arg	Asp	Leu	Lys	Asp	Tyr	
	530						535						540				
	Glu	Ala	Trp	Leu	Gly	Ile	His	Asp	Val	His	Gly	Arg	Gly	Asp	Glu	Lys	
50	545					550					555					560	
	Cys	Lys	Gln	Val	Leu	Asn	Val	Ser	Gln	Leu	Val	Tyr	Gly	Pro	Glu	Gly	
					565					570					575		
55	Ser	Asp	Leu	Val	Leu	Met	Lys	Leu	Ala	Arg	Pro	Ala	Val	Leu	Asp	Asp	
				580					585					590			

Phe Val Ser Thr Ile Asp Leu Pro Asn Tyr Gly Cys Thr Ile Pro Glu  
 595 600 605  
 5 Lys Thr Ser Cys Ser Val Tyr Gly Trp Gly Tyr Thr Gly Leu Ile Asn  
 610 615 620  
 Tyr Asp Gly Leu Leu Arg Val Ala His Leu Tyr Ile Met Gly Asn Glu  
 625 630 635 640  
 10 Lys Cys Ser Gln His His Arg Gly Lys Val Thr Leu Asn Glu Ser Glu  
 645 650 655  
 Ile Cys Ala Gly Ala Glu Lys Ile Gly Ser Gly Pro Cys Glu Gly Asp  
 660 665 670  
 15 Tyr Gly Gly Pro Leu Val Cys Glu Gln His Lys Met Arg Met Val Leu  
 675 680 685  
 Gly Val Ile Val Pro Gly Arg Gly Cys Ala Ile Pro Asn Arg Pro Gly  
 690 695 700  
 20 Ile Phe Val Arg Val Ala Tyr Tyr Ala Lys Trp Ile His Lys Ile Ile  
 705 710 715 720  
 25 Leu Thr Tyr Lys Val Pro Gln Ser Leu Lys Val Cys Leu Lys His Pro  
 725 730 735  
 Pro Ile Gln Leu Ser Phe Thr Arg Phe Gln Arg Met Trp Asn Leu Lys  
 740 745 750  
 30 Cys His Leu Gln Gln Ser Asp Asn Tyr Trp Arg Val Met Phe Val Glu  
 755 760 765  
 Ile Leu Ile Asn Val Tyr Gly Cys Phe Leu Leu Phe Cys Leu Ser Val  
 770 775 780  
 35 Leu Phe Cys Gln Cys Ser Glu Leu Arg Tyr Met Gln Val His Ile Ser  
 785 790 795 800  
 40 Arg Tyr Leu Asn Gly Leu Lys Lys His Thr Gly Ile Phe Ala Gly  
 805 810 815

45

50

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## Claims

- 5 1. A single chain protein selectively enhancing the growth of vascular endothelial cells, characterized in that it comprises the following peptide chains :

(SEQ. ID No. : 1)

10 Arg Asn Thr Ile His Glu Phe

1 5

(SEQ. ID No. : 2)

Glu Phe Gly His Glu Phe Asp Leu Tyr Glu

15 1 5 10

(SEQ. ID No. : 3)

Glu Ser Xaa Val Leu Thr Ala Arg Gln Xaa Phe Pro Ser Arg Asp Leu

20 1 5 10 15

and in that it has a molecular weight of from 72,000 to 80,000 Da when determined by SDS polyacrylamide gel electrophoresis or from 79,000 to 85,000 Da when determined under reducing conditions.

- 25 2. A process for producing the protein according to claim 1 which comprises purifying a serum-free culture supernatant of said human ovarian tumor established cell line, HUOCA-II or HUOCA-III, by combining purification techniques including (a) cation exchange chromatography, (b) heparin affinity chromatography, (c) heparin affinity high performance liquid chromatography and (d) reverse phase high performance liquid chromatography.

- 30 3. A protein of human origin which contains an amino acid sequence or a portion of the amino acid sequence represented by the following sequence (SEQ ID No. : 4) :

35 Met Trp Val Thr Lys Leu Leu Pro Ala Leu Leu Leu Gln His Val  
1 10  
Leu Leu His Leu Leu Leu Leu Pro Ile Ala Ile Pro Tyr Ala Glu  
20 30  
Gly Gln Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser  
40  
Ala Lys Thr Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile Lys  
50 60

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EP 0 550 296 A2

Thr Lys Lys Val Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr  
70  
Arg Asn Lys Gly Leu Pro Phe Thr Cys Lys Ala Phe Val Phe Asp  
80 90  
5 Lys Ala Arg Lys Lys Gln Cys Leu Trp Phe Pro Phe Asn Ser Met Ser  
100  
Ser Gly Val Lys Lys Glu Phe Gly His Glu Phe Asp Leu Tyr Glu  
110 120  
Asn Lys Asp Tyr Ile Arg Asn Cys Ile Ile Gly Lys Gly Arg Ser  
130  
10 Tyr Lys Gly Thr Val Ser Ile Thr Lys Ser Gly Ile Lys Cys Gln  
140 150  
Pro Trp Ser Ser Met Ile Pro His Glu His Ser Phe Leu Pro Ser  
160  
15 Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr Cys Arg Asn Pro  
170 180  
Arg Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser Asn Pro Glu  
190  
Val Arg Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu Val Glu  
200 210  
20 Cys Met Thr Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp His  
220  
Thr Glu Ser Gly Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro  
230 240  
His Arg His Lys Phe Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe  
250  
25 Asp Asp Asn Tyr Cys Arg Asn Pro Asp Gly Gln Pro Arg Pro Trp  
260 270  
Cys Tyr Thr Leu Asp Pro His Thr Arg Trp Glu Tyr Cys Ala Ile  
280  
30 Lys Thr Cys Ala Asp Asn Thr Met Asn Asp Thr Asp Val Pro Leu  
290 300

---

Glu Thr Thr Glu Cys Ile Gln Gly Gln Gly Glu Gly Tyr Arg Gly  
310  
35 Thr Val Asn Thr Ile Trp Asn Gly Ile Pro Cys Gln Arg Trp Asp  
320 330  
Ser Gln Tyr Pro His Glu His Asp Met Thr Pro Glu Asn Phe Lys  
340  
Cys Lys Asp Leu Arg Glu Asn Tyr Cys Arg Asn Pro Asp Gly Ser  
350 360  
40 Glu Ser Pro Trp Cys Phe Thr Thr Asp Pro Asn Ile Arg Val Gly  
370  
Tyr Cys Ser Gln Ile Pro Asn Cys Asp Met Ser His Gly Gln Asp  
380 390  
Cys Tyr Arg Gly Asn Gly Lys Asn Tyr Met Gly Asn Leu Ser Gln  
400  
45 Thr Arg Ser Gly Leu Thr Cys Ser Met Trp Asp Lys Asn Met Glu  
410 420  
Asp Leu His Arg His Ile Phe Trp Glu Pro Asp Ala Ser Lys Leu  
430  
50 Asn Glu Asn Tyr Cys Arg Asn Pro Asp Asp Asp Ala His Gly Pro  
440 450  
Trp Cys Tyr Thr Gly Asn Pro Leu Ile Pro Trp Asp Tyr Cys Pro  
460



Ile Ser Arg Cys Glu Gly Asp Thr Thr Pro Thr Ile Val Asn Leu  
 470 480  
 Asp His Pro Val Ile Ser Cys Ala Lys Thr Lys Gln Leu Arg Val  
 490  
 5 Val Asn Gly Ile Pro Thr Arg Thr Asn Ile Gly Trp Met Val Ser  
 500 510  
 Leu Arg Tyr Arg Asn Lys His Ile Cys Gly Gly Ser Leu Ile Lys  
 520  
 10 Glu Ser Trp Val Leu Thr Ala Arg Gln Cys Phe Pro Ser Arg Asp  
 530 540  
 Leu Lys Asp Tyr Glu Ala Trp Leu Gly Ile His Asp Val His Gly  
 550  
 Arg Gly Asp Glu Lys Cys Lys Gln Val Leu Asn Val Ser Gln Leu  
 560 570  
 15 Val Tyr Gly Pro Glu Gly Ser Asp Leu Val Leu Met Lys Leu Ala  
 580  
 Arg Pro Ala Val Leu Asp Asp Phe Val Ser Thr Ile Asp Leu Pro  
 590 600  
 Asn Tyr Gly Cys Thr Ile Pro Glu Lys Thr Ser Cys Ser Val Tyr  
 610  
 20 Gly Trp Gly Tyr Thr Gly Leu Ile Asn Tyr Asp Gly Leu Leu Arg  
 620 630  
 Val Ala His Leu Tyr Ile Met Gly Asn Glu Lys Cys Ser Gln His  
 640  
 His Arg Gly Lys Val Thr Leu Asn Glu Ser Glu Ile Cys Ala Gly  
 650 660  
 25 Ala Glu Lys Ile Gly Ser Gly Pro Cys Glu Gly Asp Tyr Gly Gly  
 670  
 Pro Leu Val Cys Glu Gln His Lys Met Arg Met Val Leu Gly Val  
 680 690  
 30 Ile Val Pro Gly Arg Gly Cys Ala Ile Pro Asn Arg Pro Gly Ile  
 700  
 Phe Val Arg Val Ala Tyr Tyr Ala Lys Trp Ile His Lys Ile Ile  
 710 720  
 Leu Thr Tyr Lys Val Pro Gln Ser 728

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4. A pharmaceutical composition which contains the protein of claim 1 or 3 as an active ingredient.
5. A DNA fragment which contains a nucleotide sequence or a portion of the nucleotide sequence below (SEQ ID No. : 5):

40

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ATG TGG GTG ACC AAA CTC CTG CCA GCC CTG CTG CTG CAG CAT  
 1  
 GTC CTC CTG CAT CTC CTC CTG CTC CCC ATC GCC ATC CCC TAT  
 45 45  
 GCA GAG GGA CAA AGG AAR AGA AGA AAT ACA ATT CAT GAA TTC  
 93  
 AAA AAA TCA GCA AAG ACT ACC CTA ATC AAA ATA GAT CCA GCA  
 141  
 CTG AAG ATA AAA ACC AAA AAA GTG AAT ACT GCA GAC CAA TGT  
 199  
 GCT AAT AGA TGT ACT AGG AAT AAA GGA CTT CCA TTC ACT TGC  
 237

AAG GCT TTT GTT TTT GAT AAA GCA AGA AAA CAA TGC CTC TGG  
 285  
 TTC CCC TTC AAT AGC ATG TCA AGT GGA GTG AAA AAA GAA TTT  
 333  
 5 GGC CAT GAA TTT GAC CTC TAT GAA AAC AAA GAC TAC ATT AGA  
 AAC TGC ATC ATT GGT AAA GGA CGC AGC TAC AAG GGA ACA GTA  
 381  
 TCT ATC ACT AAG AGT GGC ATC AAA TGT CAG CCC TGG AGT TCC  
 429  
 10 ATG ATA CCA CAC GAA CAC AGC TTT TTG CCT TCG AGC TAT CGG  
 477  
 GGT AAA GAC CTA CAG GAA AAC TAC TGT CGA AAT CCT CGA GGG  
 525  
 15 GAA GAA GGG GGA CCC TGG TGT TTC ACA AGC AAT CCA GAG GTA  
 573  
 CGC TAC GAA GTC TGT GAC ATT CCT CAG TGT TCA GAA GTT GAA  
 621  
 TGC ATG ACC TGC AAT GGG GAG AGT TAT CGA GGT CTC ATG GAT  
 699  
 20 CAT ACA GAA TCA GGC AAG ATT TGT CAG CGC TGG GAT CAT CAG  
 ACA CCA CAC CGG CAC AAA TTC TTG CCT GAA AGA TAT CCC GAC  
 717  
 25 AAG GGC TTT GAT GAT AAT TAT TGC CGC AAT CCC GAT GGC CAG  
 765  
 CCG AGG CCA TGG TGC TAT ACT CTT GAC CCT CAC ACC CGC TGG  
 813  
 GAG TAC TGT GCA ATT AAA ACA TGC GCT GAC AAT ACT ATG AAT  
 861  
 30 GAC ACT GAT GTT CCT TTG GAA ACA ACT GAA TGC ATC CAA GGT  
 909  
 CAA GGA GAA GGC TAC AGG GGC ACT GTC AAT ACC ATT TGG AAT  
 957  
 GGA ATT CCA TGT CAG CGT TGG GAT TCT CAG TAT CCT CAC GAG  
 1005  
 35 CAT GAC ATG ACT CCT GAA AAT TTC AAG TGC AAG GAC CTA CGA  
 GAA AAT TAC TGC CGA AAT CCA GAT GGG TCT GAA TCA CCC TGG  
 1053  
 40 TGT TTT ACC ACT GAT CCA AAC ATC CGA GTT GGC TAC TGC ICC  
 1101  
 CAA ATT CCA AAC TGT GAT ATG TCA CAT GGA CAA GAT TGT TAT  
 1149  
 CGT GGG AAT GGC AAT AAT TAT ATG GGC AAC TTA TCC CAA ACA  
 1197  
 45 AGA TCT GGA CTA ACA TGT TCA ATG TGG GAC AAG AAC ATG GAA  
 1245  
 GAC TTA CAT CGT CAT ATC TTC TGG GAA CCA GAT GCA AGT AAG  
 1293  
 CTG AAT GAG AAT TAC TGC CGA AAT CCA GAT GAT GAT GCT CAT  
 1341  
 50 GGA CCC TGG TGC TAC ACG GGA AAT CCA CTC ATT CCT TGG GAT  
 TAT TGC CCT ATT TCT CGT TGT GAA GGT GAT ACC ACA CCT ACA  
 1389  
 55 ATA GTC AAT TTA GAC CAT CCC GTA ATA TCT TGT GCC AAA ACG  
 1437  
 AAA CAA TTG CGA GTT GTA AAT GGG ATT CCA ACA CGA ACA AAC  
 1485

ATA GGA TGG ATG GTT AGT TTG AGA TAC AGA AAT AAA CAT ATC  
 1533  
 TGC GGA GGA TCA TTG ATA AAG GAG AGT TGG GTT CTT ACT GCA  
 1581  
 5 CGA CAG TGT TTC CCT TCT CGA CAC TTG AAA GAT TAT GAA GCT  
 1629  
 TGG CTT GGA ATT CAT GAT GTC CAC GGA AGA GGA GAT GAG AAA  
 1677  
 10 TGC AAA CAG GTT CTC AAT GTT TCC CAG CTG GTA TAT GCC CCT  
 GAA GGA TCA GAT CTG GTT TTA ATG AAG CTT GCC AGG CCT GCT  
 1725  
 GTC CTG GAT GAT TTT GTT AGT ACG ATT GAT TTA CCT AAT TAT  
 1773  
 15 GGA TGC ACA ATT CCT GAA AAG ACC AGT TGC AGT GTT TAT GGC  
 1821  
 TGG GGC TAC ACT GGA TTG ATC AAC TAT GAT GGC CTA TTA CGA  
 1869  
 20 GTG GCA CAT CTC TAT ATA ATG GGA AAT GAG AAA TGC AGC CAG  
 1917  
 CAT CAT CGA GGG AAG GTG ACT CTG AAT GAG TCT GAA ATA TGT  
 1965  
 GCT GGG GCT GAA AAG ATT GGA TCA GGA CCA TGT GAG GGG GAT  
 2013  
 25 TAT GGT GGC CCA CTT GTT TGT GAG CAA CAT AAA ATG AGA ATG  
 GTT CTT GGT GTC ATT GTT CCT GGT CGT GGA TGT GCC ATT CCA  
 2061  
 30 AAT CGT CCT GGT ATT TTT GTC CGA GTA GCA TAT TAT GCA AAA  
 2109  
 TGG ATA CAC AAA ATT ATT TTA ACA TAT AAG GTA CCA CAG TCA  
 2157  
 TAG 2187

wherein at least one base may be substituted based on the degeneracy of genetic code.

6. A single chain protein having an activity to enhance the growth of vascular endothelial cells obtainable from the DNA fragment of claim 5.
7. A DNA fragment complementary to the DNA fragment of claim 5.
8. An expression vector which contains the DNA fragment of claim 5.
9. A transformant transformed with the DNA fragment of claim 5.
10. A transformant transformed with the expression vector of claim 8.

FIG. 1

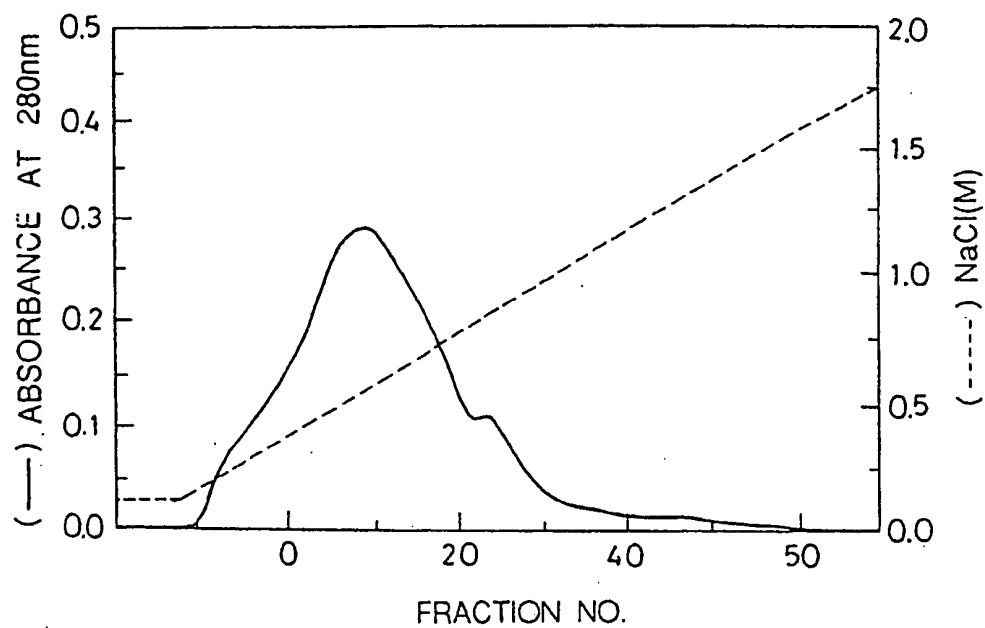


FIG. 2

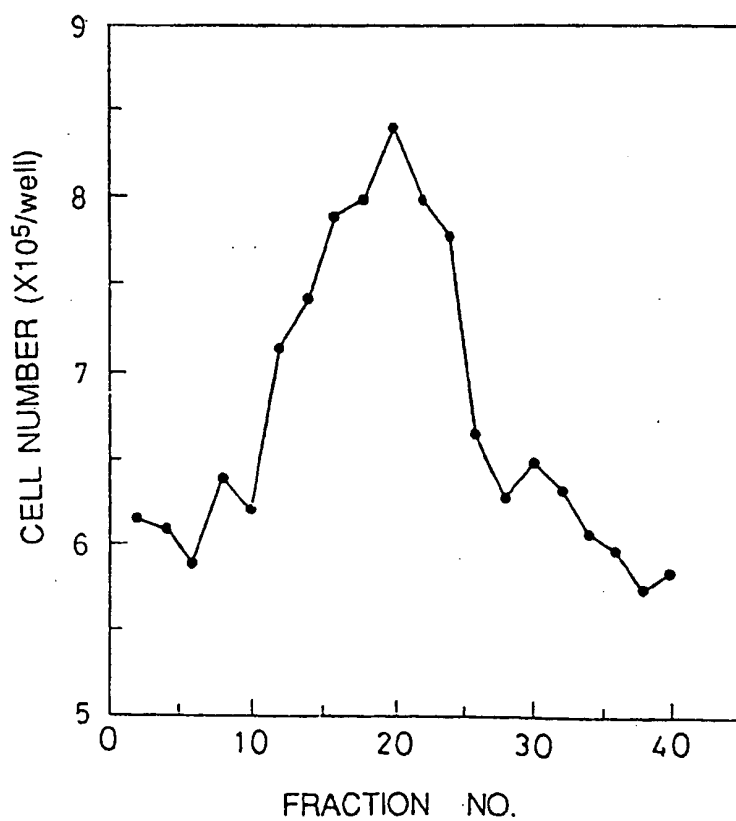


FIG. 3

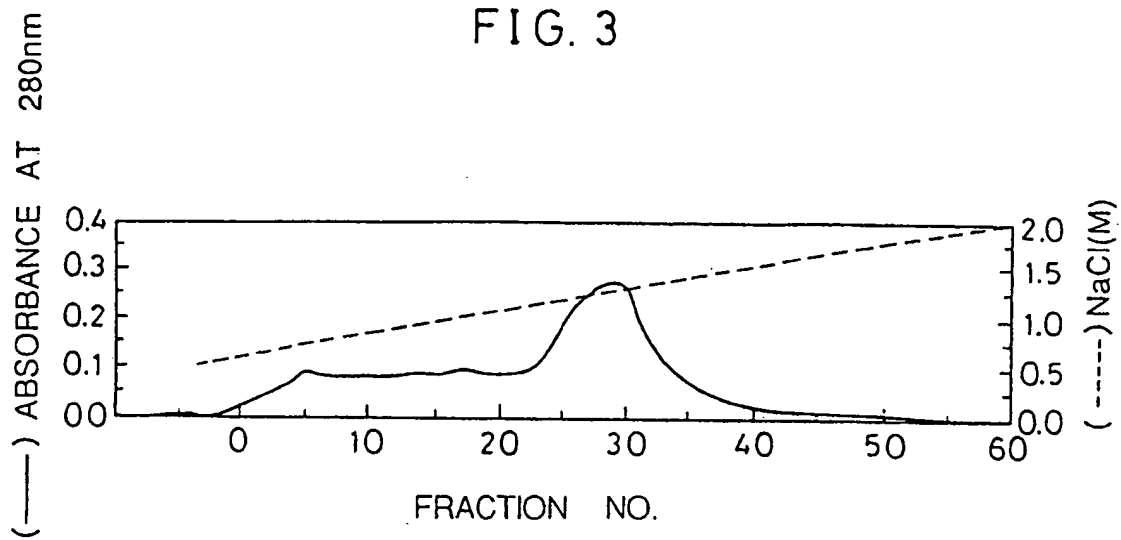


FIG. 4

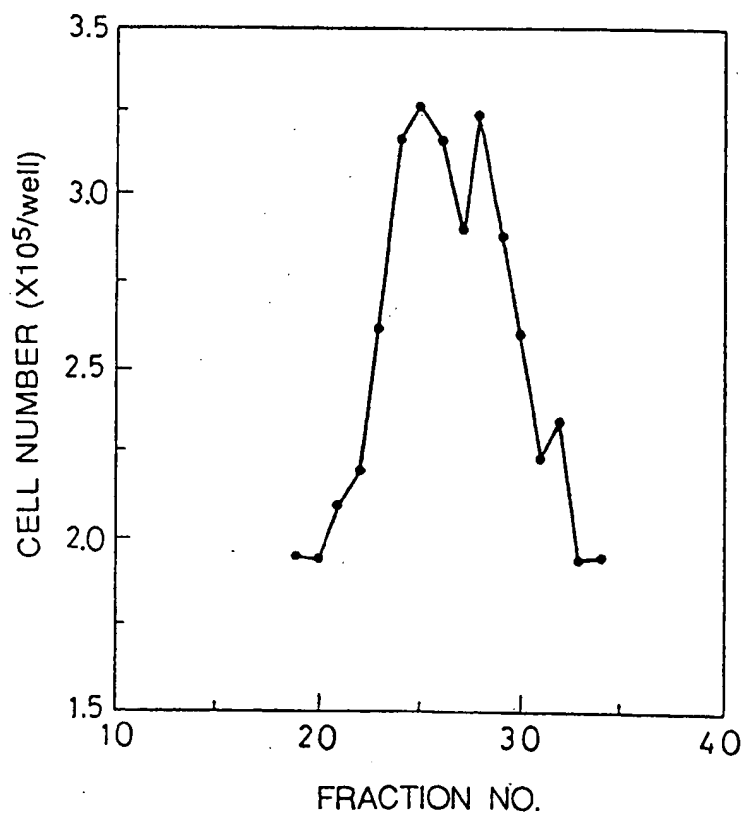


FIG.5

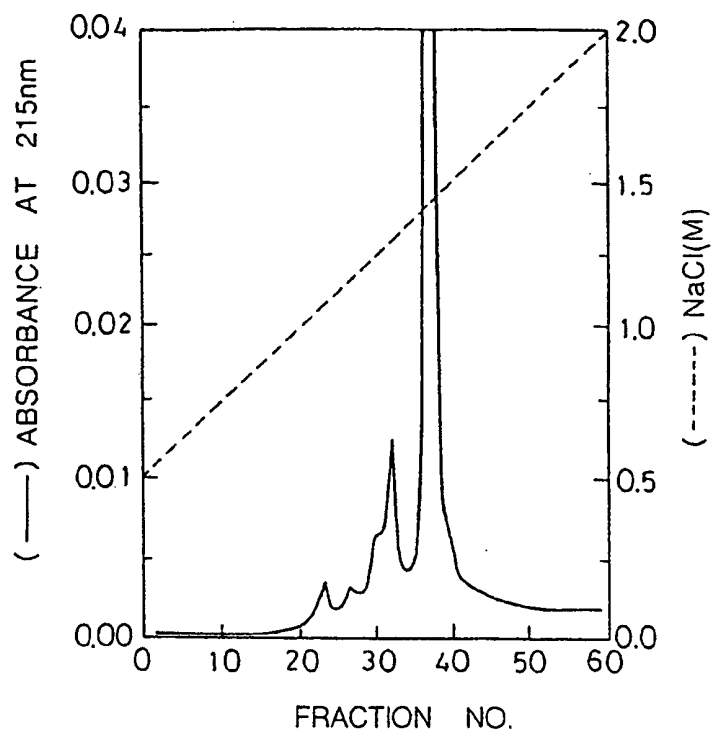


FIG.6

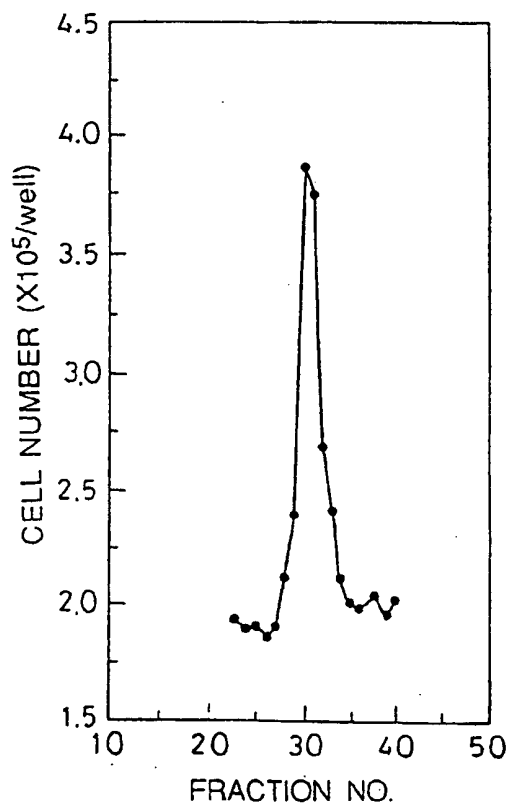


FIG. 7

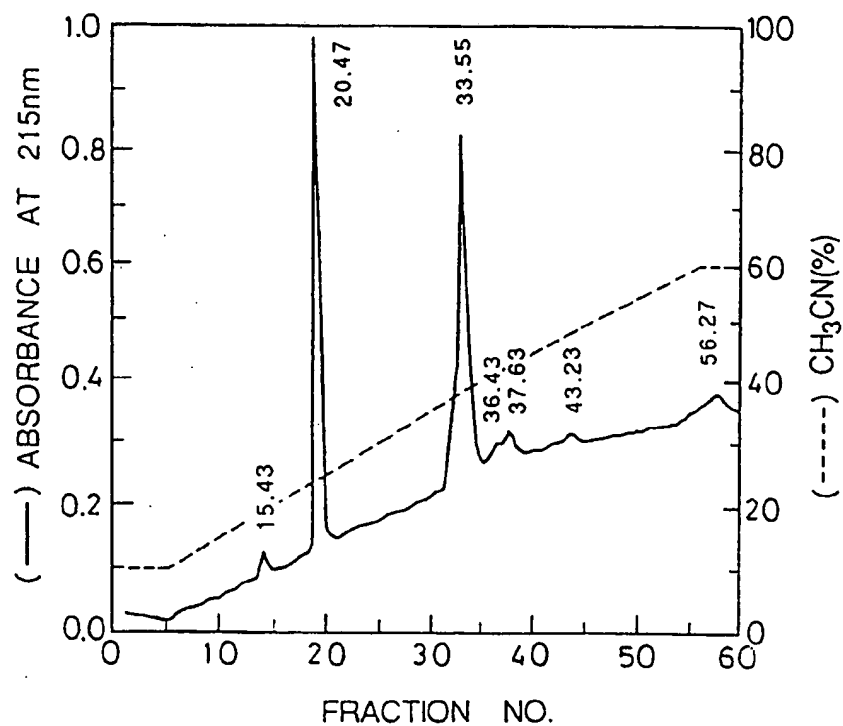


FIG. 8

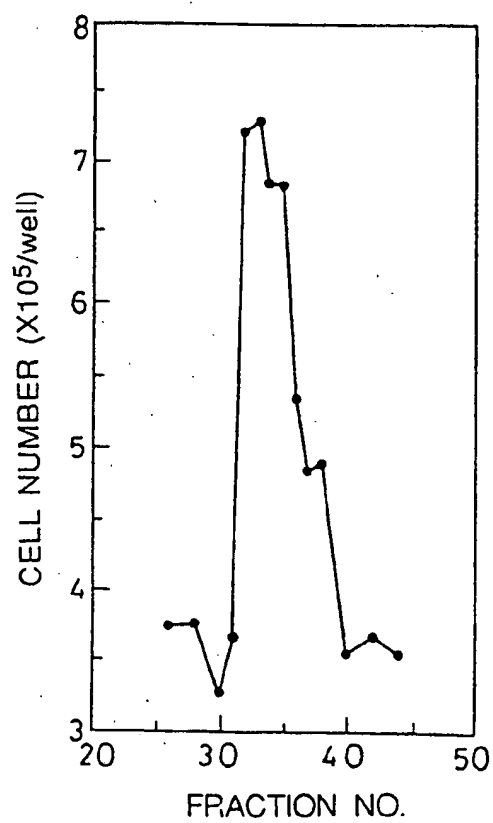


FIG. 9

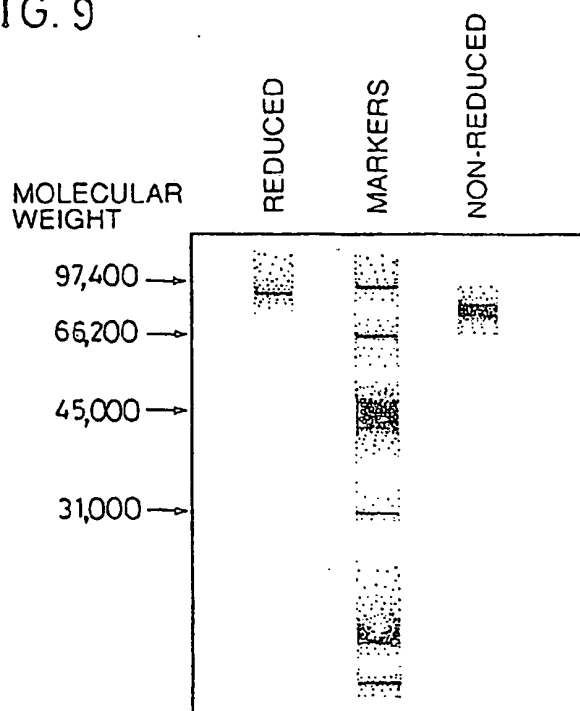


FIG. 10

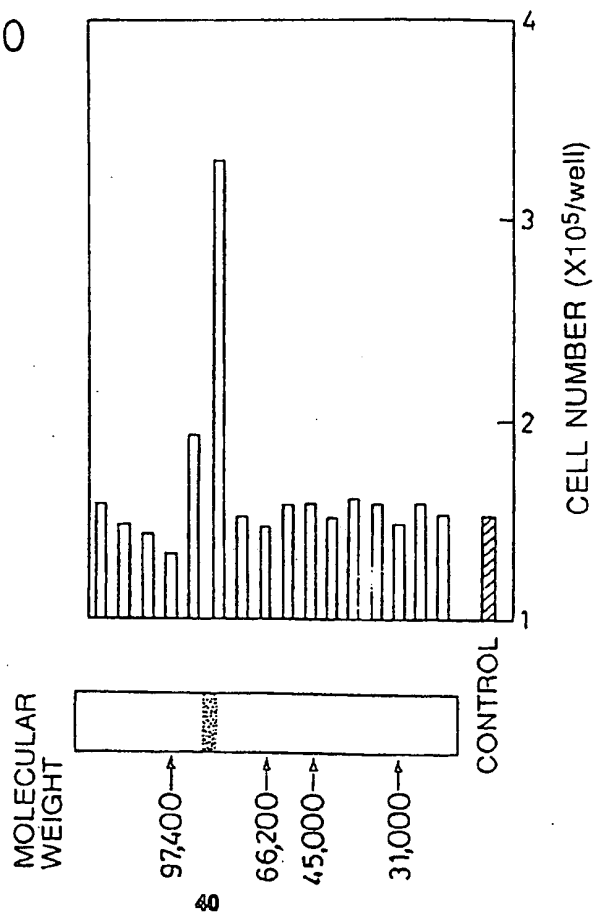




FIG. 11

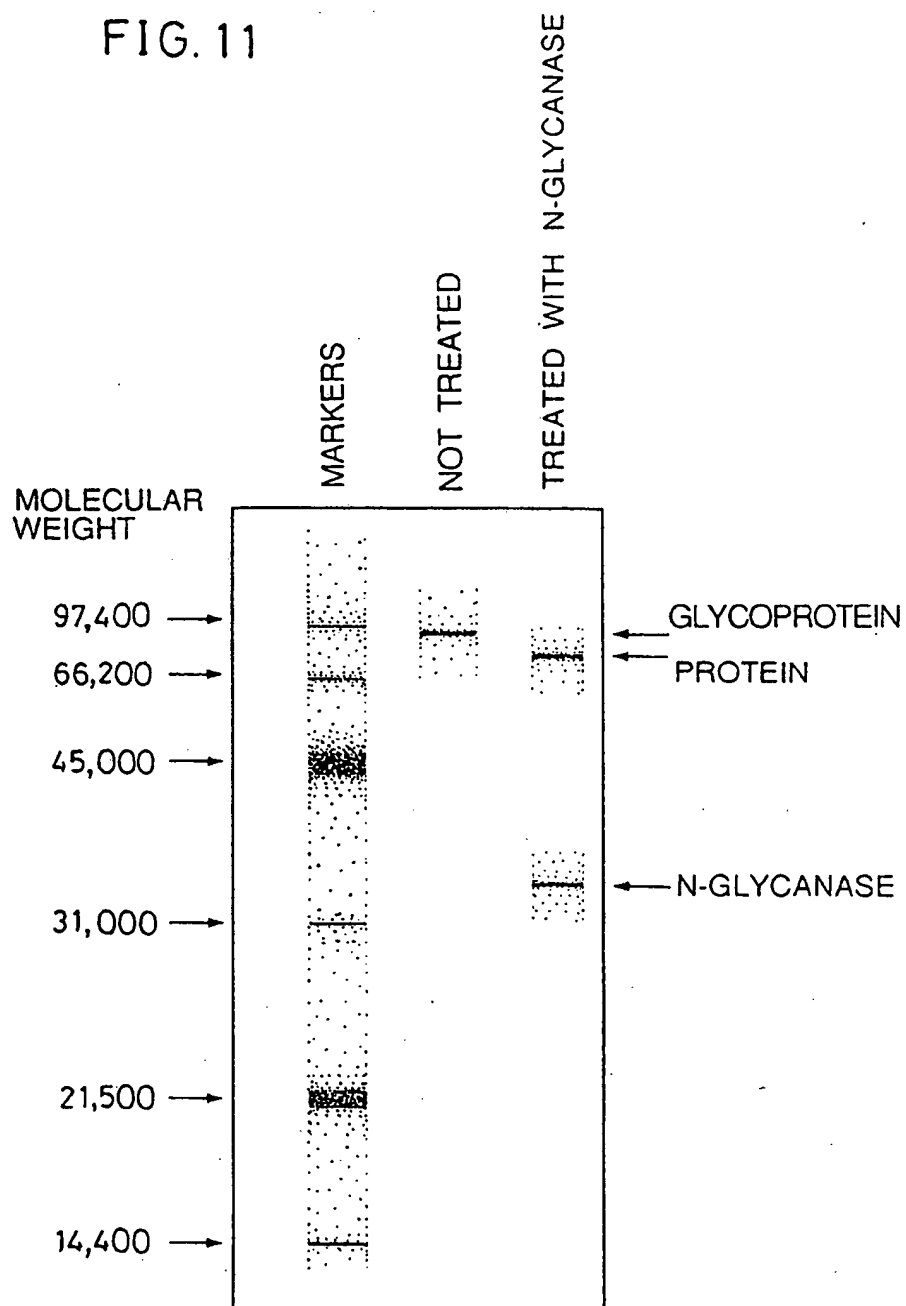


FIG. 12

1	GG	GCU	CAG	AGC	CGA	CUG	GCU	CUU	UUA	GGC	ACU	GAC	UCC	GAA	CAG	GAU	4
48	UCU	UUC	ACC	CAG	GCA	UCU	CCU	CCA	GAG	GGA	UCC	GCC	AGC	CCG	UCC	AGC	9
1			Met	Trp	Val	Thr	Lys	Leu	Leu	Pro	Ala	Leu	Leu	Leu	Gln	His	1
96	AGC	ACC	AUG	UGG	GUG	ACC	AAA	CUC	CUG	CCA	GCC	CUG	CUG	CUG	CAG	CAU	14
15	Val	Leu	Leu	His	Leu	Leu	Leu	Leu	Pro	Ile	Ala	Ile	Pro	Tyr	Ala	Glu	3
144	GUC	CUC	CUG	CAU	CUC	CUC	CUG	CUC	CCC	AUC	GCC	AUC	CCC	UAU	GCA	GAG	19
31	Gly	Gln	Arg	Lys	Arg	Arg	Asn	Thr	Ile	His	Glu	Phe	Lys	Lys	Ser	Ala	4
192	GGA	CAA	AGG	AAA	AGA	AGA	AAU	ACA	AUU	CAU	GAA	UUC	AAA	AAA	UCA	GCA	23
47	Lys	Thr	Thr	Leu	Ile	Lys	Ile	Asp	Pro	Ala	Leu	Lys	Ile	Lys	Thr	Lys	6
240	AAG	ACU	ACC	CUA	AUC	AAA	AUA	GAU	CCA	GCA	CUG	AAG	AUA	AAA	ACC	AAA	28
63	Lys	Val	Asn	Thr	Ala	Asp	Gln	Cys	Ala	Asn	Arg	Cys	Thr	Arg	Asn	Lys	7
288	AAA	GUG	AAU	ACU	GCA	GAC	CAA	UCU	GCU	AAU	AGA	UGU	ACU	AGG	AAU	AAA	33
79	Gly	Leu	Pro	Phe	Thr	Cys	Lys	Ala	Phe	Val	Phe	Asp	Lys	Ala	Arg	Lys	9
336	GGA	CUU	CCA	UUC	ACU	UGC	AAG	GCU	UUU	GUU	UUU	GAU	AAA	GCA	AGA	AAA	38
95	Gln	Cys	Leu	Trp	Phe	Pro	Phe	Asn	Ser	Met	Ser	Ser	Gly	Val	Lys	Lys	11
384	CAA	UGC	CUC	UGG	UUC	CCC	UUC	AAU	AGC	AUG	UCA	AGU	GGA	GUG	AAA	AAA	43
111	Glu	Phe	Gly	His	Glu	Phe	Asp	Leu	Tyr	Glu	Asn	Lys	Asp	Tyr	Ile	Arg	12
432	GAA	UUU	GGC	CAU	GAA	UUU	GAC	CUC	UAU	GAA	AAC	AAA	GAC	UAC	AUU	AGA	47
127	Asn	Cys	Ile	Ile	Gly	Lys	Gly	Arg	Ser	Tyr	Lys	Gly	Thr	Val	Ser	Ile	14
480	AAC	UGC	AUC	AUU	GGU	AAA	GGA	CGC	AGC	UAC	AAG	GGA	ACA	GUA	UCU	AUC	52
143	Thr	Lys	Ser	Gly	Ile	Lys	Cys	Gln	Pro	Trp	Ser	Ser	Met	Ile	Pro	His	15
528	ACU	AAG	AGU	GGC	AUC	AAA	UGU	CAG	CCC	UGG	AGU	UCC	AUG	AUA	CCA	CAC	57
159	Glu	His	Ser	Phe	Leu	Pro	Ser	Ser	Tyr	Arg	Gly	Lys	Asp	Leu	Gln	Glu	17
576	GAA	CAC	AGC	UUU	UUG	CCU	UCG	AGC	UAU	CGG	GGU	AAA	GAC	CUA	CAG	GAA	62
175	Asn	Tyr	Cys	Arg	Asn	Pro	Arg	Gly	Glu	Glu	Gly	Gly	Pro	Trp	Cys	Phe	19
624	AAC	UAC	UGU	CGA	AAU	CCU	CGA	GGG	GAA	GAA	GGG	GGA	CCC	UGG	UGU	UUC	67
191	Thr	Ser	Asn	Pro	Glu	Val	Arg	Tyr	Glu	Val	Cys	Asp	Ile	Pro	Gln	Cys	20
672	ACA	AGC	AAU	CCA	GAG	GUA	CGC	UAC	GAA	GUC	UGU	GAC	AUU	CCU	CAG	UGU	71
207	Ser	Glu	Val	Glu	Cys	Met	Thr	Cys	Asn	Gly	Glu	Ser	Tyr	Arg	Gly	Leu	22
720	UCA	GAA	GUU	GAA	UGC	AUG	ACC	UGC	AAU	GGG	GAG	AGU	UAU	CGA	GGU	CUC	76
223	Met	Asp	His	Thr	Glu	Ser	Gly	Lys	Ile	Cys	Gln	Arg	Trp	Asp	His	Gln	23
788	AUG	GAU	CAU	ACA	GAA	UCA	GGC	AAG	AUU	UGU	CAG	CGC	UGG	GAU	CAU	CAG	81

FIG. 12 (cont.)

239	Thr	Pro	His	Arg	His	Lys	Phe	Leu	Pro	Glu	Arg	Tyr	Pro	Asp	Lys	Gly	25
816	ACA	CCA	CAC	CGG	CAC	AAA	UUC	UUG	CCU	GAA	AGA	UAU	CCC	GAC	AAG	GGC	86
255	Phe	Asp	Asp	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Gln	Pro	Arg	Pro	Trp	27
864	UUU	GAU	GAU	AAU	UAU	UGC	CGC	AAU	CCC	GAU	GGC	CAG	CCG	AGG	CCA	UGG	91
271	Cys	Tyr	Thr	Leu	Asp	Pro	His	Thr	Arg	Trp	Glu	Tyr	Cys	Ala	Ile	Lys	28
912	UGC	UAU	ACU	CUU	GAC	CCU	CAC	ACC	CGC	UGG	GAG	UAC	UGU	GCA	AUU	AAA	95
287	Thr	Cys	Ala	Asp	Asn	Thr	Met	Asn	Asp	Thr	Asp	Val	Pro	Leu	Glu	Thr	30
960	ACA	UGC	GCU	GAC	AAU	ACU	AUG	AAU	GAC	ACU	GAU	GUU	CCU	UUG	GAA	ACA	100
303	Thr	Glu	Cys	Ile	Gln	Gly	Gln	Gly	Glu	Gly	Tyr	Arg	Gly	Thr	Val	Asn	31
1008	ACU	GAA	UGC	AUC	CAA	GGU	CAA	GGA	GAA	GGC	UAC	AGG	GGC	ACU	GUC	AAU	105
319	Thr	Ile	Trp	Asn	Gly	Ile	Pro	Cys	Gln	Arg	Trp	Asp	Ser	Gln	Tyr	Pro	33
1056	ACC	AUU	UGG	AAU	GGA	AUU	CCA	UGU	CAG	CGU	UGG	GAU	UCU	CAG	UAU	CCU	110
335	His	Glu	His	Asp	Met	Thr	Pro	Glu	Asn	Phe	Lys	Cys	Lys	Asp	Leu	Arg	35
1104	CAC	GAG	CAU	GAC	AUG	ACU	CCU	GAA	AAU	UUC	AAG	UGC	AAG	GAC	CUA	CGA	115
351	Glu	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Ser	Glu	Ser	Pro	Trp	Cys	Phe	36
1152	GAA	AAU	UAC	UGC	CGA	AAU	CCA	GAU	GGG	UCU	GAA	UCA	CCC	UGG	UGU	UUU	119
367	Thr	Thr	Asp	Pro	Asn	Ile	Arg	Val	Gly	Tyr	Cys	Ser	Gln	Ile	Pro	Asn	38
1200	ACC	ACU	GAU	CCA	AAC	AUC	CGA	GUU	GGC	UAC	UGC	UCC	CAA	AUU	CCA	AAC	124
383	Cys	Asp	Met	Ser	His	Gly	Gln	Asp	Cys	Tyr	Arg	Gly	Asn	Gly	Lys	Asn	39
1248	UGU	GAU	AUG	UCA	CAU	GGA	CAA	GAU	UGU	UAU	CGU	GGG	AAU	GGC	AAA	AAU	129
399	Tyr	Met	Gly	Asn	Leu	Ser	Gln	Thr	Arg	Ser	Gly	Leu	Thr	Cys	Ser	Met	41
1296	UAU	AUG	GGC	AAC	UUA	UCC	CAA	ACA	AGA	UCU	GGA	CUA	ACA	UGU	UCA	AUG	134
415	Trp	Asp	Lys	Asn	Met	Glu	Asp	Leu	His	Arg	His	Ile	Phe	Trp	Glu	Pro	43
1344	UGG	GAC	AAG	AAC	AUG	GAA	GAC	UUA	CAU	CGU	CAU	AUC	UUC	UGG	GAA	CCA	139
431	Asp	Ala	Ser	Lys	Leu	Asn	Glu	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Asp	Asp	44
1392	GAU	GCA	AGU	AAG	CUG	AAU	GAG	AAU	UAC	UGC	CGA	AAU	CCA	GAU	GAU	GAU	143
447	Ala	His	Gly	Pro	Trp	Cys	Tyr	Thr	Gly	Asn	Pro	Leu	Ile	Pro	Trp	Asp	46
1440	GCU	CAU	GGA	CCC	UGG	UGC	UAC	ACG	GGA	AAU	CCA	CUC	AUU	CCU	UGG	GAU	148
463	Tyr	Cys	Pro	Ile	Ser	Arg	Cys	Glu	Gly	Asp	Thr	Thr	Pro	Thr	Ile	Val	47
1488	UAU	UGC	CCU	AUU	UCU	CGU	UGU	GAA	GGU	GAU	ACC	ACA	CCU	ACA	AUA	GUC	153
479	Asn	Leu	Asp	His	Pro	Val	Ile	Ser	Cys	Ala	Lys	Thr	Lys	Gln	Leu	Arg	49
1536	AAU	UUA	GAC	CAU	CCC	GUA	AUA	UCU	UGU	GCC	AAA	ACG	AAA	CAA	UUG	CGA	158
495	Val	Val	Asn	Gly	Ile	Pro	Thr	Arg	Thr	Asn	Ile	Gly	Trp	Met	Val	Ser	51
1584	GUU	GUA	AAU	GGG	AUU	CCA	ACA	CGA	ACA	AAC	AUA	GGA	UGG	AUG	GUU	AGU	163
511	Leu	Arg	Tyr	Arg	Asn	Lys	His	Ile	Cys	Gly	Gly	Ser	Leu	Ile	Lys	Glu	52
1632	UUG	AGA	UAC	AGA	AAU	AAA	CAU	AUC	UGC	GGA	GGA	UCA	UUG	AUA	AAG	GAG	167
527	Ser	Trp	Val	Leu	Thr	Ala	Arg	Gln	Cys	Phe	Pro	Ser	Arg	Asp	Leu	Lys	54
1680	AGU	UGG	GUU	CUU	ACU	GCA	CGA	CAG	UGU	UUC	CCU	UCU	CGA	GAC	UUG	AAA	172
543	Asp	Tyr	Glu	Ala	Trp	Leu	Gly	Ile	His	Asp	Val	His	Gly	Arg	Gly	Asp	55
1728	GAU	UAU	GAA	GCU	UGG	CUU	GGA	AUU	CAU	GAU	GUC	CAC	GGA	AGA	GGA	GAU	177
559	Glu	Lys	Cys	Lys	Gln	Val	Leu	Asn	Val	Ser	Gln	Leu	Val	Tyr	Gly	Pro	57
1776	GAG	AAA	UGC	AAA	CAG	GUU	CUC	AAU	GUU	UCC	CAG	CUG	GUA	UAU	GGC	CCU	182
575	Glu	Gly	Ser	Asp	Leu	Val	Leu	Met	Lys	Leu	Ala	Arg	Pro	Ala	Val	Leu	59
1824	GAA	GGA	UCA	GAU	CUG	GUU	UUA	AUG	AAG	CUU	GCC	AGG	CCU	GCJ	GUC	CUG	187

FIG. 12 (cont.)

591	Asp	Asp	Phe	Val	Ser	Thr	Ile	Asp	Leu	Pro	Asn	Tyr	Gly	Cys	Thr	Ile	60
1872	GAU	GAU	UUU	GUU	AGU	ACG	AUU	GAU	UUA	CCU	AAU	UAU	GGA	UGC	ACA	AUU	191
607	Pro	Glu	Lys	Thr	Ser	Cys	Ser	Val	Tyr	Gly	Trp	Gly	Tyr	Thr	Gly	Leu	62
1920	CCU	GAA	AAG	ACC	AGU	UGC	AGU	GUU	UAU	GGC	UGG	GGC	UAC	ACU	GGA	UUG	196
623	Ile	Asn	Tyr	Asp	Gly	Leu	Leu	Arg	Val	Ala	His	Leu	Tyr	Ile	Met	Gly	63
1968	AUC	AAC	UAU	GAU	GGC	CUA	UUA	CGA	GUG	GCA	CAU	CUC	UAU	AUA	AUG	GGA	201
639	Asn	Glu	Lys	Cys	Ser	Gln	His	His	Arg	Gly	Lys	Val	Thr	Leu	Asn	Glu	65
2016	AAU	GAG	AAA	UGC	AGC	CAG	CAU	CAU	CGA	GGG	AAG	GUG	ACU	CUG	AAU	GAG	206
655	Ser	Glu	Ile	Cys	Ala	Gly	Ala	Glu	Lys	Ile	Gly	Ser	Gly	Pro	Cys	Glu	67
2064	UCU	GAA	AUA	UGU	GCU	GGG	GCU	GAA	AAG	AUU	GGA	UCA	GGA	CCA	UGU	GAG	211
671	Gly	Asp	Tyr	Gly	Gly	Pro	Leu	Val	Cys	Glu	Gln	His	Lys	Met	Arg	Met	68
2112	GGG	GAU	UAU	GGU	GGC	CCA	CUU	GUU	UGU	GAG	CAA	CAU	AAA	AUG	AGA	AUG	215
687	Val	Leu	Gly	Val	Ile	Val	Pro	Gly	Arg	Gly	Cys	Ala	Ile	Pro	Asn	Arg	70
2160	GUU	CUU	GGU	GUC	AUU	GUU	CCU	GGU	CGU	GGA	UGU	GCC	AUU	CCA	AAU	CGU	220
703	Pro	Gly	Ile	Phe	Val	Arg	Val	Ala	Tyr	Tyr	Ala	Lys	Trp	Ile	His	Lys	71
2208	CCU	GGU	AUU	UUU	GUC	CGA	GUA	GCA	UAU	UAU	GCA	AAA	UGG	AUA	CAC	AAA	225
719	Ile	Ile	Leu	Thr	Tyr	Lys	Val	Pro	Gln	Ser	***	Leu	Lys	***	Val	Cys	73
2256	AUU	AUU	UUA	ACA	UAU	AAG	GUA	CCA	CAG	UCA	UAG	CUG	AAG	UAA	GUG	UGU	230
735	Leu	Lys	His	Pro	Pro	Ile	Gln	Leu	Ser	Phe	Thr	***	Arg	Phe	Gln	Arg	75
2304	CUG	AAG	CAC	CCA	CCA	AUA	CAA	CUG	UCU	UUU	ACA	UGA	AGA	UUU	CAG	AGA	235
751	Met	Trp	Asn	Leu	Lys	Cys	His	Leu	Gln	Gln	Ser	***	Asp	Asn	Tyr	Trp	76
2352	AUG	UGG	AAU	UUA	AAA	UGU	CAC	UUA	CAA	CAA	UCC	UAA	GAC	AAC	UAC	UGG	239
767	Arg	Val	Met	Phe	Val	Glu	Ile	Leu	Ile	Asn	Val	Tyr	Gly	Cys	Phe	Leu	78
2400	AGA	GUC	AUG	UUU	GUU	GAA	AUU	CUC	AUU	AAU	GUU	UAU	GGG	UGU	UUU	CUG	244
783	Leu	Phe	Cys	Leu	Ser	Val	Leu	Phe	Cys	Gln	Cys	***	Ser	Glu	Leu	Arg	79
2448	UUG	UUU	UGU	UUG	UCA	GUG	UUA	UUU	UGU	CAA	UGU	UGA	AGU	GAA	UUA	AGG	249
799	Tyr	Met	Gln	Val	***	***	His	Ile	Ser	***	Arg	Tyr	Leu	Asn	Gly	Leu	81
2496	UAC	AUG	CAA	GUG	UAA	UAA	CAU	AUC	UCC	UGA	AGA	UAC	UUG	AAU	GGA	UUA	254
815	Lys	Lys	His	Thr	Gly	Ile	Phe	Ala	Gly	***	***						82
2544	AAA	AAA	CAC	ACA	GGU	AUA	UUU	GCU	GGA	UGA	UAA						257